IMMUNOHISTOCHEMICAL EXPRESSION OF SURVIVIN IN NORMAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

DISSERTATION

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Branch VI

ORAL PATHOLOGY AND MICROBIOLOGY

(2012 – 2015)

CERTIFICATE

Certified that the dissertation entitled: **"Immunohistochemical expression of survivin in normal mucosa, oral epithelial dysplasia and oral squamous cell carcinoma"** is a bonafide record of the work done by Dr.George Jacob under our guidance during his post graduate study during the period of 2012-2015 under THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI, in partial fulfilment for the degree of MASTER OF DENTAL SURGERY IN ORAL PATHOLOGY AND MICROBIOLOGY, BRANCH VI. It has not been submitted (partial or full) for the award of any other degree or diploma.

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Dr.George Jacob

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
APES	3-amino Propyl Triethoxy Silane
Bax	Bcl-2 associated X protein
bFGF	Basic fibroblast growth factor
Bcl-2	B-cell lymphoma-2
BIR	Baculovirus IAP Repeat
cIAP1	Cellular Inhibitor of Apoptosis 1
cIAP2	Cellular Inhibitor of Apoptosis 2
Cdk4	cyclin-dependent kinase 4
DAB	3-diamino benzidine tetra hydrochloride
DIABLO	Direct IAP Binding Protein with low pl
DNA	Deoxy Ribo Nucleic Acid
DPX	Distrene Dibutylpthalate in Xylene
ED	Epithelial Dysplasia
EDTA	Ethylene Diamine Tetra Acetic Acid
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme Linked Immunosorbent Assay
HSILs	High-Grade Squamous Intraepithelial Lesions
HCC	Hepatocellular Carcinoma
HPV	Human Papilloma Virus
HRP	Horse Radish Peroxidase
hTERT	Human Telomerase Reverse Transcriptase
Hsp90	Heat Shock Protein 90
IAP	Inhibitor of Apoptosis Protein
IHC	Immunohistochemistry
INCENP	Inner Centromere Proteins

LSILs	Low-grade Squamous Intraepithelial Lesions
ML-IAP	Melanoma Inhibitor of Apoptosis Protein
mRNA	Messenger Ribonuclei Acid
NFκB	Nuclear Factor κ B
NAIP	Neuronal Apoptosis Inhibitory Protein
OSCC	Oral Squamous Cell Carcinoma
OED	Oral Epithelial Dysplasia
PARP	Poly ADP-Ribose Polymerase
PCR	Polymerase Chain Reaction
PDGF	Platelet Derived Growth Factor
Smac	Second Mitochondria-derived Activator of Caspases
SMC	Smooth Muscle Cells
SPSS	Statistical Package for Social Sciences
SCC	Squamous Cell Carcinoma
TNM	Tumor Node Metastasis
TRIS	Tris hydroxyl methyl aminomethane
Ts-XIAP	Testis-specific X Inhibitor of Apoptosis Protein
UV block	Ultra Violet block
VEGF	Vascular Endothelial Growth Factor
XIAP	X chromosome binding Inhibitor of Apoptosis Protein

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ABSTRACT

BACKGROUND:

Oral squamous cell carcinoma (OSCC) is the most frequent malignant tumour of the oral cavity. It is accepted that screening for oral squamous cell carcinomas and oral premalignant lesions may decrease the devastating morbidity and mortality associated with the disease. This has lead to widespread research for identification of molecular based bio-markers. Among them survivin is a recently characterised IAP protein which is a member of the inhibitor of apoptosis family. Further investigation of survivin during tumour growth and progression may yield important insights into its functional role in carcinogenesis.

OBJECTIVES:

To compare the expression of survivin in oral epithelial dysplasias and oral squamous cell carcinoma with that in normal mucosa.

MATERIALS AND METHODS:

Study subjects consisted of formalin fixed paraffin embedded blocks of histologically confirmed cases of oral epithelial dysplasia (n=30), oral squamous cell carcinoma (OSCC) (n=30) and normal mucosa (n=30) in the age group of 20-70

years. Immunohistochemical staining was performed on 4µm sections of paraffin embedded sections with the use of survivin rabbit monoclonal antibody (PathnSitu).

RESULTS:

There was statistical significance between the expression of survivin among oral epithelial dysplasia, oral squamous cell carcinoma and normal mucosa with a p value of 0.001 (Kruskal-Wallis test significant at 0.01 level).

INTEPRETATION AND CONCLUSION:

Results showed that there is significant up regulation of survivin expression in oral epithelial dysplasias and oral squamous cell carcinoma when compared to that in normal mucosa. It is concluded that survivin which is an inhibitor of apoptosis protein can be identified as a useful tool for the identification of precancerous lesions at higher risk for progression into invasive carcinoma.

KEYWORDS:

Squamous cell carcinoma; oral epithelial dysplasia; survivin; inhibitor of apoptosis; immunohistochemistry.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most frequent malignant tumour of the oral cavity (90% of all tumours).[1] OSCC is an aggressive malignant cancer, with high mortality and morbidity, which commonly occurs in middle-aged male and older individuals.[2,3] This kind of cancer has been strongly correlated with specific risk factors, such as tobacco and/or alcohol use.[4] In spite of the therapeutic advances, the 5-year survival time remains at about 55%.[2,5]

It is generally accepted that cancer development in the oral mucosa may be preceded by an identifiable but non-invasive precursor lesion.[1] However, it is still uncertain how many oral squamous cell carcinomas (OSCC) arise from precursor lesions and how many develop from apparently normal oral mucosa. Previous studies have shown that between 16% and 62% of OSCC are associated with oral leukoplakia, the best known oral pre-cancerous lesion.[6] Furthermore, OSCC can also arise from other pre-cancerous oral lesions and conditions, such as verrucous hyperplasia[7], submucous fibrosis[8] and lichen planus[9]. Oral epithelial dysplasia, not associated with any specific clinical appearance, is a term assigned to the histopathological changes associated with increased risk of malignant transformation. The presence of epithelial dysplasia is generally regarded as one of the most important predictors of malignant transformation in pre-malignant lesions .[6, 10, 11]

Carcinogenesis is the process by which normal cells undergo malignant transformation, following several genetic and epigenetic alterations. The cancer starts when the cells present uncontrolled proliferation.[12] Cell division is a physiological process that occurs in almost all tissues and under many circumstances. The balance between proliferation and programmed cell death (apoptosis) is kept by tight

regulation between both processes. The transition of normal epithelium to invasive cancer is progressive and accompanied by "multiple hits" which promote proliferation, angiogenesis, local invasion and eventually distant metastasis.[13]

The malignant process involves DNA damage, leading to changes of the production and/or function of certain proteins. These changes may occur by failures during the replication of DNA, or by deficiencies of its repair engine. DNA alterations responsible for cancer usually occur in genes that regulate the cell cycle, growth and differentiation, which are classified into oncogenes and tumor suppressor genes. The oncogenes are originated from proto-oncogenes, these latter genes are involved in basic mechanisms of normal cell growth regulation and include growth factors, receptors for growth factors, signal transducers and nuclear transcription factors. Most of the oncogenes implicated in other cancer types also contribute to OSCC.[13]

Although up to a third (3-33%) of oral precancerous lesions will evolve into invasive OSCC over a 10 year interval, no reliable histopathological parameters have been identified that predict their potential for subsequent transformation. A surgical management is often impractical, especially in patients with multiple and extensive precancerous lesions.[1] Novel molecular predictors of malignant progression are needed to identify oral precancerous lesions at greater risk of invasive transformation.[1]

Several molecular markers implicated in the carcinogenesis of OSCC have been evaluated by many investigators, including molecules involved in the cell cycle regulation, apoptosis, angiogenesis, DNA repair system and degradation of extracellular matrix. Identifying these molecules by analysis of DNA, RNA and

protein expression might permit the early diagnosis, beyond to predict better therapeutic alternatives. The most used techniques have been cDNA microarray, PCR, in situ hybridization, immunohistochemistry (IHC), ELISA and western-blot, among others. However, the available evidence about the molecular markers still remains inconclusive. Accordingly, there is a continuous need to define the most important biological markers for OSCC progression and invasiveness.[2]

Much importance has been given to apoptotic markers which are expressed in both premalignant and malignant lesions. Among them, survivin is a recently characterised protein which is found expressed in solid and haematological malignancies.[1] Survivin, a unique member in the IAP (inhibitor of apoptosis protein) family, is undetectable in most normal adult tissues but is highly expressed in cancer. It is cell cycle regulated, and is involved in both control of apoptosis (or programmed cell death) and regulation of cell division.[14, 15, 16, 17]

Further investigation of survivin and other apoptotic inhibitors during tumor growth and progression may yield important therapeutic strategies for combating cancer. The present study is therefore designed to analyse the immunohistochemical expression of Survivin in oral epithelial dysplasias and oral squamous cell carcinoma.

AIMS & OBJECTIVES

AIMS:

- To analyse the immunohistochemical expression of survivin in normal oral mucosa.
- To analyse the immunohistochemical expression of survivin in oral epithelial dysplasias.
- To analyse the immunohistochemical expression of survivin in oral squamous cell carcinoma
- To evaluate statistically the immunohistochemical expression of survivin in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma
- To compare the expression of survivin in oral epithelial dysplasias and oral squamous cell carcinoma to that of normal mucosa.

OBJECTIVES:

Apoptosis plays a major role during embryonic development and in the maintenance of tissue homeostasis. Deregulation of apoptosis resulting in reduced cell death is thought to participate in cancer by facilitating additional transforming mutations. Survivin, a unique member in the IAP family, is undetectable in most normal adult tissues but is over expressed in cancers. Immunohistochemistry is been used to investigate the potential role of survivin in identification of precancerous lesions at higher risk of progression into invasive carcinoma. This study was done to immunohistochemically analyse the expression of survivin in tissue sections of oral epithelial dysplasia and oral squamous cell carcinoma and to compare them with normal oral mucosa.

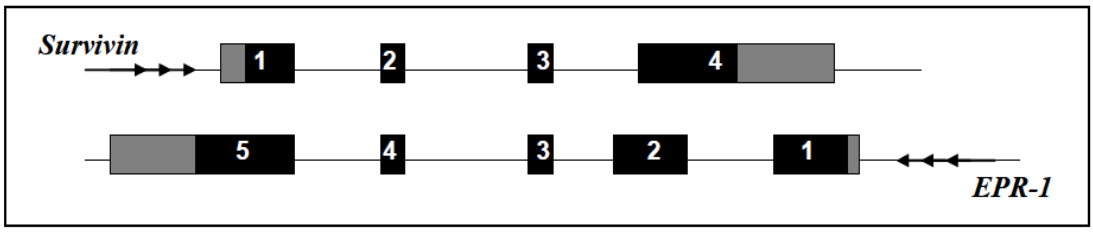
REVIEW OF LITERATURE

SURVIVIN

Apoptosis is an evolutionary conserved process of autodestruction that is initiated by cells in response to different stimuli, including UV radiation, toxins, hormones, and cytokines.[18] Apoptosis is activated to eliminate cells carrying highly damaged DNA, whereas it is inhibited to preserve important cell functions.[16] Apoptotic machinery has a major role in both normal conditions and diseased tissues, including skin.[19] To maintain epidermal homeostasis, the apoptotic process is finely regulated by a number of antiapoptotic molecules, including Akt and NF-kB.[20] NF-kB induces the transcription of antiapoptotic genes such as the Bcl-2 and IAP (inhibitor of apoptosis) families.[2,5] Survivin, a unique member in the IAP family, is undetectable in most normal adult tissues but is highly expressed in cancer.[1]

DISCOVERY:

In 1997 Survivin was first cloned and delineated in Diego Altieri's laboratory at Yale University School of Medicine. During that time, the Altieri laboratory was reviewing the coagulation cascade and their contribution to vascular injury. They were in an attempt to elucidate the mechanism by which activation of the coagulation cascade through protease factor Xa binding to its receptor effector cell protease-1 (EPR-1) promoted thrombin formation as well as cellular growth. To elucidate other genes homologous to EPR-1, hybridization screening of the human P1 genomic library with the cDNA of EPR-1 was done and they identified a new gene in the same locus but on the DNA strand opposite of EPR-1.[21,22]



Map of the survivin gene:Survivin and EPR-1 genes located on opposite strands of chromosome 17q25 locus. Arrows indicate the direction of transcription, numbered boxes represent exons, gray shades indicate the un-translated regions (Altieri DC 1995)

Sequence analysis of this new gene anticipated the development of a unique 1.9kb transcript that would form a 142 amino acid protein, unrelated to EPR-1, with a molecular weight of 16,389kD which were identified in transformed lymphoid cell lines by blotting techniques. Blast analysis of the protein sequence registered the presence of abaculovirus IAP repeat (BIR) domain that is the differentiating element of the IAP inhibitor of the apoptosis family, thus the new protein was inferred to function as a survival protein and was given the name Survivin.[23]

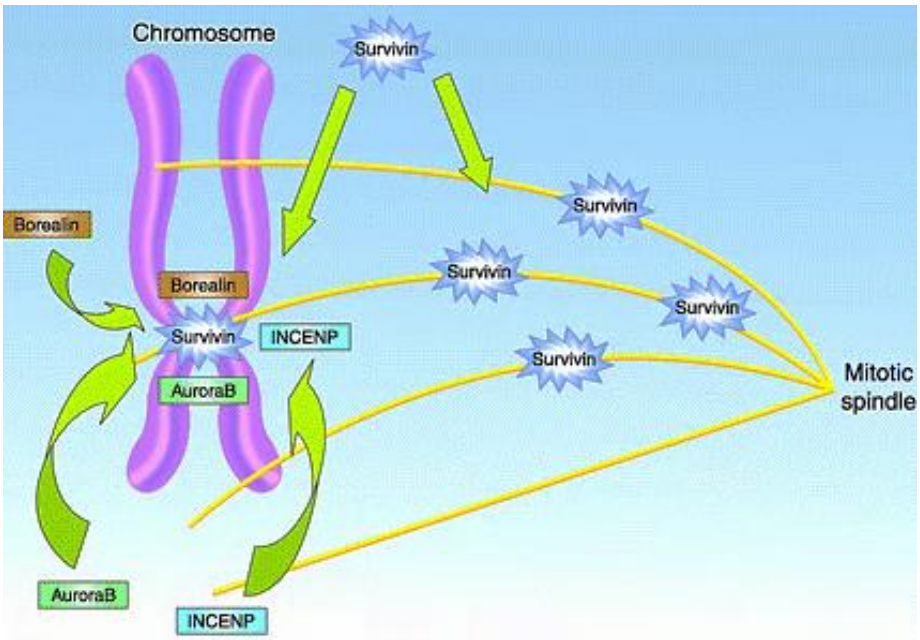
PROTEIN STRUCTURE OF SURVIVIN:

The structure of human survivin, as determined by X-ray crystallography shows an amino-terminal globular zinc finger BIR domain and a long carboxy-terminal helix separated by a short linker segment. The amino terminal comprises of three alpha helices (residues 14-21, 31-41, 68-80) and three beta-sheets (residues 43-45, 55-58, 61-64) which simulate the BIR domain that is conserved in the IAP family.[24]

There are four variants namely survivin–2α, survivin-2B, survivin-ΔEx3,survivin-3B.[25,26,27] The former two are located in the cytoplasm whereas the latter is located in the nucleus. It is proposed that the localization in distinct cellular compartments of different nuclear-cytoplasmic versions might constitute a regulatory mechanism for the activity of different splice variants of survivin. The diverse isoforms of survivin is considered to play specific roles in neoplasia which may be partially determined by their differential nuclear-cytoplasmic transport and localization.[28]

ROLE OF SURVIVIN IN CELL DIVISION:

During the cell cycle, survivin is first detected on centromeres and functions in a narrow time window at metaphase and anaphase and localizes to two main subcellular pools. The first pool of survivin is directly linked with polymerized tubulin. This pool comprises centrosomes, microtubules of the metaphase and anaphase spindle and the remnants of the mitotic apparatus, and implies an organization of microtubule dynamics. The second pool of survivin confines to the kinetochores of metaphase chromosomes. In this pool, survivin is associated with regulators of cytokinesis, such as Aurora B kinase, Inner centromere protein (INCENP) and Borealin/Dasra , which supports a role for survivin as a subunit of the chromosomal passenger complex that is essential for proper chromosome segregation and cytokinesis.[29] Thus survivin contributes to bipolar spindle formation by mediating the proper targeting of chromosomal passenger proteins to kinetochores and stabilizing the microtubules.[30]

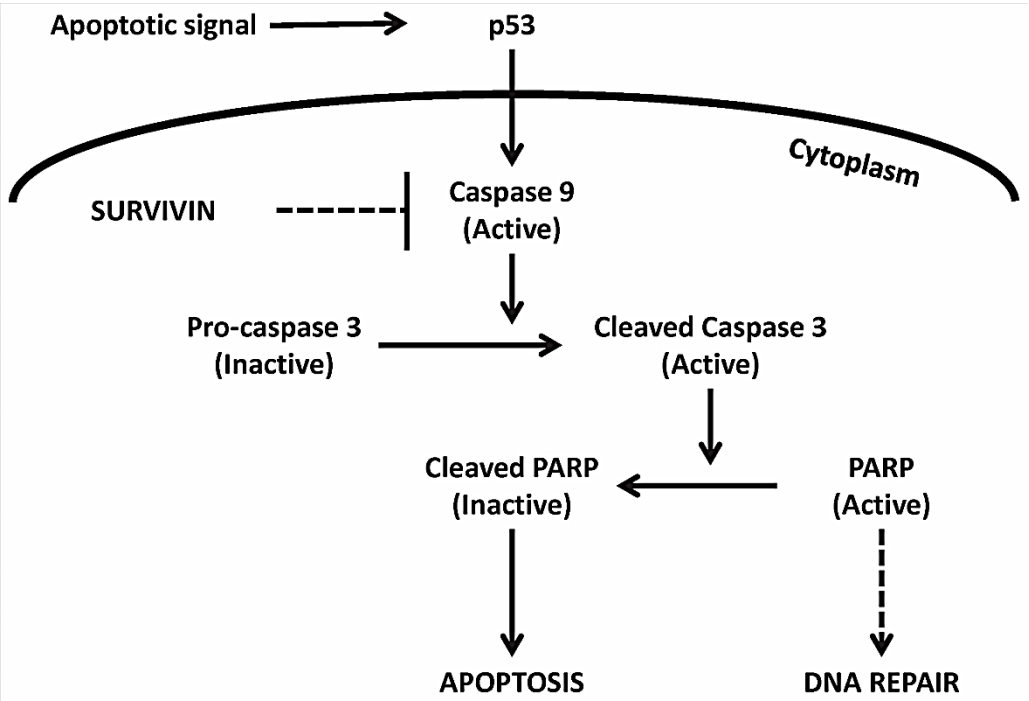


Function of survivin in mitosis. Survivin is a component of the chromosomal passenger complex that is essential for proper chromosome segregation and cytokinesis. Another pool of survivin is directly associated with polymerized tubulin and contributes to the regulation of microtubule dynamics (Mita AC 2008)

Any disruption of survivin will result in cell division defects. Homozygous knockout of the survivin gene in mouse embryonic stem cells resulted in the disarrayed microtubule formation and polyploidy which caused early embryonic lethality.[31] This suggests that survivin and INCENP function in the same pathways during cell cycle progression.[15] Aurora-B is a mitotic kinase that plays an important role in chromosome segregation and cytokinesis. Survivin is also reported to bind Aurora-B and to enhance the phosphorylating activity of Aurora-B toward its substrates.[32] Thus, survivin functions jointly with other passenger proteins such as INCENP and Aurora-B to regulate cell division.[15]

THE MECHANISM OF INHIBITION OF APOPTOSIS BY SURVIVIN:

Survivin regulates apoptosis by directly inhibiting the caspases responsible for induction and execution of apoptosis or by indirectly inhibiting caspase function by regulating Smac/Diablo.[15] The direct action of survivin is by binding to and inhibiting caspase 9 leading to deactivation of the apoptotic pathway. Therefore procaspase 3 remains uncleaved and hence PARP (Poly ADP-ribose polymerase) also is uncleaved and hence the latter remains active and persists with DNA repair, resulting in the inhibition of apoptosis.[33]

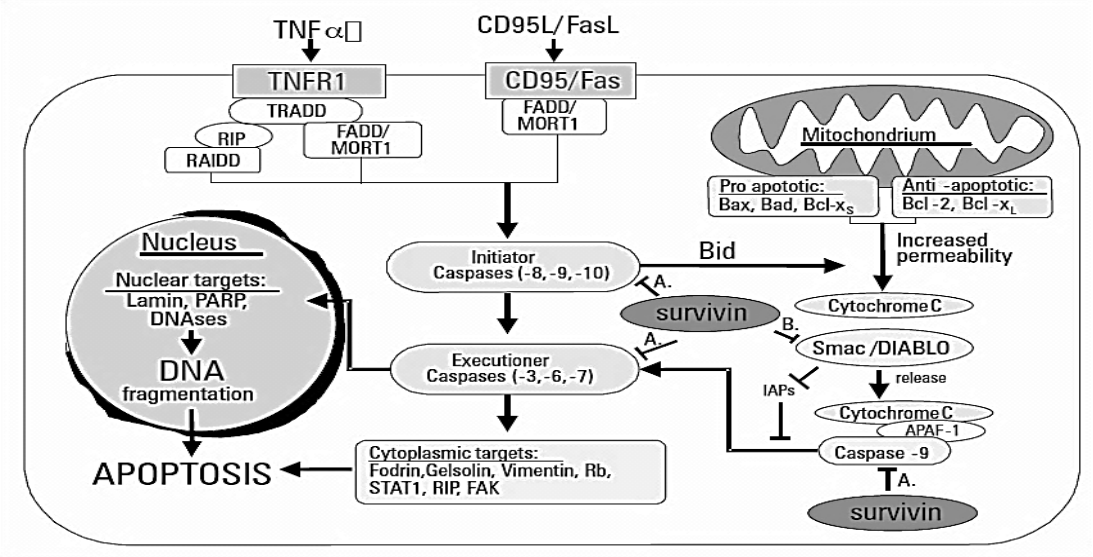


Survivin inhibition pathway: Survivin binds to and inhibits caspase 9, caspase 9 is unable to cleave caspase 3, caspase 3 is unable to cleave PARP, PARP promotes DNA repair and does not induce apoptosis. (Malhotra 2013)

Interaction of survivin with caspases 3, 7, and 9 requires the phosphorylation of survivin at threonine34. A mutation from this threonine to alanine (T34A) can induce the release of cytochrome *c* from the mitochondria culminating in apoptosis. CDK1, a cyclin-dependent kinasephoshorylates survivin during mitosis, suggesting a direct physical interaction between the two for phosphorylation of survivin.[34]

The hepatocytes of heterozygous survivin knockout mice have decreased levels of activated procaspase-3, procaspase-8, procaspase-9 and Bid but increased susceptibility to Fas induced apoptosis. Fas induced apoptosis is associated with release of cytochrome c and up-regulation of survivin in the nucleus, cytosol and mitochondria.[35] Upon Fas stimulation, survivin interacts with Cdk4, which releases p21 from its complex with Cdk4 which in turn lets p21 complex with caspase-3, which leads to its inactivation within the mitochondria.[15,36]

Second mitochondria-derived activator of caspases (Smac/DIABLO) can promote apoptosis by binding to and suppressing the inhibitory effects of the IAP proteins. Smac is released from mitochondria into the cytosol along with cytochrome c during execution of the mitochondrial apoptosis pathway.[37] Ectopic over expression of Smac increases caspase-3 activity, and down regulates the activity of survivin.[38] Survivin may inhibit caspase activity indirectly via binding to and sequestering Smac, thus inhibiting Smac binding to other IAPs.



EXPRESSION OF SURVIVIN IN FETAL TISSUES AND NORMAL ADULT TISSUES:

Several studies has shown the expression of survivin in the fetal lung, heart, liver, kidney and gastrointestinal tract and in fetal tissues where apoptosis occurs, such as the stem cell layers of stratified epithelia, pancreas thymic medulla and endocrine glands.[39] Survivin is also considered to be a key mediator of embryonic submandibular salivary gland epithelial cell survival.[40]

The expression of survivin in normal human adult tissues appeared only in a few published reports, contrary to numerous reports examining the role of survivin in cancer.[15] However, its expression has been reported in a few normal growing adult human tissues, including thymus **(Kobayashi et al, 2002)**[41], colonic mucosa **(Gianani et al, 2001)**[42], placenta **(Shiozaki et al, 2003; Lehner et al, 2001)**[43,44], bone marrow **(Altieri & Marchisio, 1999)** [45] and keratinocytes of the basal layer of the skin. **(Grossman et al, 2001; O'Driscoll et al, 2003; Chiodino et al, 1999)**[46,47,48]

Survivin expression is detected in oligodendrocytes, astrocytes, neurons, ependymal cells and choroid plexus in the human brain. Conditional survivin deletion in neuronal precursor cells showed apoptosis in retina, cerebrum, cerebellum, brainstem and spinal cord signifying the survivin as an antiapoptotic protein in neuronal development.[49,50]

Survivin is expressed in the adult liver tissues and is down regulated by ischemia but up-regulated by hepatectomy. Furthermore, the Fas agonistic antibody Jo2 induces survivin expression in liver whereas survivin haplo-insuffciency sensitizes hepatocytes to Jo2 antibody– mediated apoptosis, indicating that hepatocyte proliferation and apoptosis are regulated by survivin.[35,51,52]

Survivin is expressed in gastrointestinal tract mucosa, which suggests that survivin may be important in regulating self-renewal and differentiation of crypt stem cells.[15,17] Survivin expression has also been reported in melanocytes, keratinocytes, testes and ovary. Stem cell factor and human chorionic gonadotropin also induce survivin expression in testes and ovarian granulosa cells, suggesting that survivin may have a role in the regulation of spermatogenesis and oogenesis.[48,53,54,55]

EXPRESSION OF SURVIVIN IN HEMATOPOIETIC AND IMMUNE CELLS:

Role of Survivin facilitating angiogenesis:

Following exposure to angiogenic factors such as Vascular endothelial growth factor (VEGF) and Basic fibroblast growth factor (bFGF) an elevated expression of survivin was noted in cultured vascular endothelial cells.[56] The mechanism by which survivin promotes angiogenesis by preserving microtubule structure integrity and

inhibiting apoptosis in endothelial cells, which is necessary for the viability and cytoprotection of the cells.[57,58]

If endothelial cells are transfected with genes encoding survivin-specific siRNA or phosphorylation-defective form of survivin, vascular regression during tumor angiogenesis occurs.[59,60] PDGF stimulation in smooth muscle cells (SMC) causes up-regulation of survivin which promotes cell viability while disruption of survivin function interrupts neointimal formation in mouse femoral arteries following injury.[61] Changes in cell death or viability in SMCs have been implicated in diseases of vascular remodeling.[62]

Role of Survivin in Polymorphonuclear Neutrophils & T-Lymphocytes:

Mature blood neutrophils do not express survivin whereas immature neutrophils express.[63] Under inflammatory conditions or stimulation with the neutrophil growth factor/granulocyte macrophage colony-stimulating factor, terminally differentiated neutrophils can re-express survivin. Contrarily, the life span of the neutrophils is shortened even in the presence of granulocyte colony stimulating factor or interleukin-3 with the administration of antisense survivin oligonucleotides. Increased survivin expression in mature cells by these cytokines without cell cycle progression, illustrate that survivin expression is not confined to proliferating cells and that survivin can block apoptosis in a cell cycle–independent manner.[64]

Phytohemagglutinin,interleukin-2 plus anti-CD3 or concanavalin A can induce the expression of survivin in thymocytes, peripheral T lymphocytes, and splenic T cells.[17] In experimental animals with survivin deletions occurring at different stages of

T-cell development, a defect in thymic development/ decreased the number of peripheral blood T cells was observed.[65]

Survivin in erythropoiesis & thrombopoiesis:

Survivin is differentially expressed during erythroid and megakaryocyte development.[66] Survivin is expressed in maturing erythroid cells whereas murine megakaryocytes expressed reduced levels of survivin. Over expression of survivin in murine bone marrow cells caused decreased production of megakaryocytes and arrested their terminal maturation. Conversely haplo-insufficiency of the survivin gene lowered erythroid cell expansion without affecting megakaryocytes. Survivin deficiency impaired production of erythroid or megakaryocytic colonies in vitro which signifies the role of survivin in erythropoiesis and thrombopoiesis.[17,66]

Expression of Survivin in Adult Stem Cells:

Survivin is expressed in normal human CD34+ cells that contain the population of stem cells capable of long-term hematopoietic reconstitution.[17] Hematopoietic growth factors such as stem cell factor, Flt3 ligand, and thrombopoietin which stimulate cell cycle progression, cell proliferation, and survival of CD34+ cells, boost the expression of survivin in these cells.[67] Discordantly, growth factor deprivation weaken survivin expression, which correlates with exalted active caspase-3 and apoptosis.[63]

Although its expression is coupled with cell cycle progression, survivin was up regulated in all phases of the cell cycle in CD34+ cells after cytokine stimulation, suggesting cell cycle-dependent and cell cycle-independent regulation, in contrast to

the cell cycle-dependent and selective expression during G2-M phase in the majority of cancer cells.[17,63]

Ectopic expression of survivin in mouse bone marrow cells intensifies the proliferation and cell cycle progression of hematopoietic progenitor cells and arrests growth factor deprivation-induced apoptosis. Antisense survivin retroviral constructs which antagonize survivin inhibited these effects. These data advocate that survivin governs hematopoietic progenitor cell proliferation.[67]

EXPRESSION OF SURVIVIN IN CANCERS:

Survivin is increasingly expressed in several cancers including central nervous system, breast, esophageal, gastric, colorectal, pancreatic, hepatocellular, lung, laryngeal, bladder, renal, uterine, ovarian and prostate cancers, as well as soft tissue sarcomas and melanoma.[17] Survivin is also highly expressed in patients with hematologic malignancies such as acute leukemias, lymphomas, and myelodysplastic syndromes.[68] Positive survivin expression in Philadelphia-positive chronic myelogenous leukemia in blast crisis, suggests that up-regulation of survivin expression is involved in development of chronic myelogenous leukemia and that survivin expression and hematopoietic cell differentiation are related.[69,70]

In cancer cells, elevated survivin is related to reduced levels of apoptosis, intensified proliferative index, resistance to chemotherapy and increased rate of tumor recurrence. Studies have shown that increased survivin expression is akin to clinicopathologic variables of aggressive disease and shows a strong correlation with shorter disease-free or overall survival, signifying the protein as an independent

prognostic indicator of poor outcome in patients with most tumor types. Elevated survivin as a powerful indicator of favorable outcome in patients with gastric cancer,non–small-cell lung cancer and osteosarcoma has been documented.[17]

The variations in prognostic value of survivin may reflect the divergence in the techniques used to detect survivin, nuclear versus cytoplasmic subcellular localization, and/or differential regulation of splice variants with opposing functions.[17] Nuclear survivin expression is an unfavorable prognostic indicator in hepatocellular, esophageal, non-small-cell lung and ovarian cancers, cholangio-carcinoma, mantel cell lymphoma and endometrial cancers.[14,71] Contrarily, favorable outcome associated with nuclear survivin has been narrated for bladder, gastricand breast cancers, osteosarcoma and ependymoma.[14]

Nuclear survivin may regulate cell proliferation whereas cytoplasmic survivin may be involved in cell survival but not cell proliferation.[14] Using immunohistochemical analysis, nuclear survivin has been detected with several polyclonal antibodies. Cytoplasmic survivin has been detected with polyclonal antibodies as well as monoclonal antibodies.[17] Predominantly nuclear survivin is detected in ovarian cancer whereas predominantly cytoplasmic survivin is detected in pancreatic cancer.[72,73] Polyclonal antibody detects predominantly cytoplasmic survivin in oral squamous carcinoma cells but detects predominantly nuclear survivin in patients with laryngeal squamous cell carcinomas.[1,74] These findings suggest that antibody specificity may not be the determinant responsible for the predictive value of survivin localization.[17]

EPITHELIAL DYSPLASIA

Alterations of the head and neck (H&N) mucosa have, for centuries, been intimately involved in the advancement of our understanding of precancers or potentially malignant lesions. Baillie (1806)[75] and his Royal Society committee in Edinburgh first proposed the concept of premalignancy in 1806 and his Royal Society committee in Edinburgh first proposed the concept of premalignancy in 1806, but the first to actually apply the concept, Sir James Paget, (1871)[76] gave it a decidedly maxillofacial focus by speculating, in 1851, that pipe smokers with 'leukokeratosis' or 'smoker's patch,' i.e. nicotine palatinus, carried an increased risk of oral cancer. The popular diagnostic term, carcinoma in situ (CIS), was coined by Broders (1932) using a laryngeal example.[77]

Warnakulasuriya S (2007)[78] at a workshop coordinated by the WHO Collaborating Centre for Oral Cancer and Precancer in the UK, issues related to terminology, definitions and classification of oral precancer were discussed by an expert group. The term, "potentially malignant disorders", was recommended to refer to precancer as it conveys that not all disorders described under this term may transform into cancer. There are varieties of premalignant / precancerous / preneoplastic / potentially malignant lesions in man which have only a small chance of ever progressing into invasive cancer. In all these cases "Progression to cancer is exception and not rule".

The gold standard for the assessment of oral potentially malignant disorders remains the microscopic evaluation of haematoxylin and eosin stained sections for the presence of epithelial dysplasia. "Epithelial dysplasia is loss in the uniformity of the individual cell as well as a loss in their architectural orientation".

These pre-neoplastic lesions are generally labeled as lesions with epithelial dysplasia. While assessing the pre-neoplastic mucosa the following features are observed:

1. Keratinization pattern
2. Thickness of the epithelium
3. Alteration in the cellular layer
4. Connective tissue changes

Of all these, alteration in the cellular layer is what generally described as epithelial dysplasia. This is further subdivided into (Oral mucosa in health and disease - A E Dolby).

A. Abnormalities related to different cell layer

- Basal cell hyperplasia
- Drop shaped rete pegs
- Loss of polarity
- Loss of cohesiveness
- Irregular epithelial stratification

B. Cytological abnormalities

- Hyperchromatic nuclei
- Anisocytosis and anisonucleiosis
- Pleomorphism of cell and nuclei

C. Cell division

- Increase in mitotic activity
- Level of mitotic activity
- Abnormal mitosis

Smith and Pindborg in 1969 laid down photographic standards which allow scoring of epithelial dysplasia, and are referred as gold standards. It includes additional criteria like:

1. Increased nuclear / cytoplasmic ratio
2. Increased cell keratinization
3. Epithelial thickness
4. Surface characteristics viz. keratotic, nonkeratotic, parakeratotic

Kramer gave importance to the connective tissue changes and studied the nature, distribution and severity of inflammatory cell infiltrate and concluded that whenever there is a shift from predominately lymphocytic infiltration to plasma cells and Russell bodies, the lesion has high chances of turning into malignancy.

Many grading system of grading epithelial dysplasia have been proposed in order to standardize the severity of dysplastic features. The various grading system put forth by different authors are as follows:

1. WHO classification (2005)
2. Brothwell D J (2003)
3. Ljubljana (2003)
4. Kuffer & Lombardi (2002)
5. Speight et al (1996)
6. Nivelle et al (1995)
7. Lumermann H et al (1995)
8. Burkhardt & Maerkar (1981)
9. WHO Classification (1978)
10. Banoczy & Csiba (1976)
11. Smith & Pindborg's photographic method (1969)

During the workshop coordinated by the WHO Collaborating Centre for Oral Cancer and Precancer the Working Group discussed the 2005 WHO classification and recommended its adaptation for wider use. Accordingly when architectural disturbance is accompanied by cytological atypia (variations in the size and shape of the keratinocytes) the term dysplasia applies. Criteria used for diagnosing oral epithelial dysplasia are listed below.

CRITERIA USED FOR DIAGNOSING DYSPLASIA

Architecture	**Cytology**
Irregular epithelial stratification	Abnormal variation of nuclear size (anisonucleosis)
Loss of polarity of basal cells	Abnormal variation of nuclear shape (nuclear pleomorphism)
Basel cell hyperplasia	Abnormal variation in cell size (anisocytosis)
Drop-shaped rete ridges	Abnormal variation in cell shape (cellular pleomorphism)
Increased number of mitotic figures	Increased nuclear cytoplasmic ratio
Abnormally superficial mitoses	Increased nuclear size
keratinization in single cells (dyskeratosis)	Atypical mitotic figures
Keratin pearls within rete ridges	Increased number & size of nuclei

Conventionally, dysplasia is divided into grades of mild, moderate and severe.

EXPRESSION OF SURVIVIN IN ORAL EPITHELIAL DYSPLASIA:

Lo Mozio et al (2003) used immunohistochemistry to investigate the potential role of survivin as an early predictor of malignant transformation in precancerous and cancerous lesions of the oral cavity. In their study survivin was present in 10/30 cases of oral precancerous lesions without malignant progression and in 15/16 cases of oral precancerous lesion that evolved into full-blown carcinomas. They concluded that

high expression of survivin is an early event during oral carcinogenesis and may provide a useful tool for identification of precancerous lesions at higher risk of progression into invasive carcinoma[1]

Tanaka et al (2003) investigated the distribution of survivin protein expression in oral squamous cell carcinomas and oral premalignant lesions. In their study, 58% of tumors and 37% of premalignant lesions examined were positive for survivin, while no immunoreaction was observed in corresponding normal tissues. They postulated that the state of survivin expression may be an important discriminator for the progression of head and neck squamous cell carcinomas. They could not find a relation between the degree of survivin expression and the grade/stage of OSCC in the study. Thus they hypothesized that survivin protein accumulation might be an early event during oral carcinogenesis, since one-third of oral pre-malignant lesions examined in this study had protein expression.[79]

Lin CY et al (2005) investigated the immunohistochemical expression of survivin in oral epithelial dysplasia and OSCC. Cytoplasmic survivin staining was detected in 97% of oral epithelial dysplasia specimens and 98% of OSCC specimens but not in adjacent normal oral mucosal tissues. The labeling index for survivin significantly increased from ED to OSCC samples. They stated that their results indicated that survivin expression may be an important early event in oral carcinogenesis and predicts unfavorable prognosis for OSCC.[80]

Oluwadayo Oluwadara et al (2009) assessed the presence of survivin in oral lichen planus, oral squamous cell carcinoma and epithelial dysplasia. The level of survivin was high in 64.3% of oral lichen planus, 96.3% of oral squamous cell

carcinoma and 100% of epithelial dysplasia. They explained that the high survivin expression in oral lichen planus heightened risk for transformation into OSCC. They proposed that oral lichen planus lesions that are in the process of transforming to OSCC present histomorphologically as oral lichen planus,but possess a certain molecular signature that represents the specific factors that drive progression to cancer.[81]

Junita Indarti et al (2013) conducted a study to identify risk factors and assess the role of survivin in predicting progressivity in precancerous cervical lesions. Among the various parameters assessed it was found that high survivin expression may predict the progressivity of precancerous cervical lesions.[82]

ORAL SQUAMOUS CELL CARCINOMA

Oral cancer is one of the ten most common cancers in the world and shows marked geographic differences in occurrence[83]. It accounts for about 3-4% of all cancers. Of all oral cancers 96% are carcinomas and 4% are sarcomas. The most common type of oral cancer is squamous cell carcinoma, constituting about 90% of oral malignancies and causing more deaths than any other oral disease.[84]

Oral cancer ranks number one among men and number three among women in India with a frequency ranging from 15% - 20% among all cancers.[85] Incidence rates are available from epidemiological surveys. Incidence rates 21.4 / 100,000; 25 / 100,000; 21 / 100,000 were reported by Wahi (1968)[86], Malaowalla et al (1976)[87], Gupta et al (1980)[88] respectively. Mehta et al (1971)[89] in an Indian study reported a prevalence rate of 0.1%.

The cause of oral squamous cell carcinoma is multifactorial. No single causative agent or factor (carcinogen) has been clearly defined or accepted. Wynder et al (1957)[90] applied the terms 'intrinsic' and 'extrinsic' to group factors, which act together to produce malignant transformation. Extrinsic factors include external agents such as tobacco, smoke, alcohol, syphilis and sunlight (lip cancer). Intrinsic factors include systemic or generalized states such as malnutrition or iron deficiency anemia. Heredity does not appear to play a major etiologic role in oral carcinoma.

The clinical features of oral cancer differ considerably for different intraoral locations. The description of this disease in various intra oral locations according to strictly defined anatomical landmarks should help to obtain reliable and comparable information on the etiological factors and the prognosis of this disease. The topographical classification proposed by Roed-Peterson and Renstrup (1969), which is recommended by WHO (1980) provides a detailed and comprehensive approach suitable for tropical countries.[91]

Oral squamous cell carcinoma has a varied clinical presentation including:

- Exophytic (mass forming; fungating, papillary, verruciform)
- Endophytic (invasive, burrowing ulcerated)
- Leukoplakic (white patch)
- Erythroplakic (red patch)
- Erythroleukoplakic (combined red and white patch)

Tumor size and extent of metastatic spread of oral squamous cell carcinoma are best indicators of the patient's prognosis. Quantification of these clinical parameters is called as staging of the disease. Clinical staging refers to an assessment of the extent of the disease before undertaking treatment and their purposes include, selection of the most appropriate treatment and meaningful comparison to the results. TNM classification was given by UICC in 1987.

Squamous cell carcinoma arises from dysplastic surface epithelium and is characterized histopathologically by invasive islands and cords of malignant squamous epithelial cells. Invasion is represented by irregular extension of lesional epithelium through the basement membrane and into sub-epithelial connective tissue. Individual squamous cells and sheets or islands of cells are seen thriving as independent entities within the connective tissue, without attachment to the surface epithelium.

Invading cells and cell masses may extend deeply into underlying adipose tissue, muscle or bone destroying the original tissue as they progress. Lesional cells may surround and destroy blood vessels and may invade into the lumina of veins or lymphatics. There is often a strong inflammatory or immune cell response and focal areas of necrosis may be present. The lesional epithelium is capable of inducing the formation of new small blood vessels (angiogenesis) and occasionally a dense fibrosis (desmoplasia or scirrhous change).

Based on proliferation and degree of differentiation lesions are graded on a three or four point scale. The less differentiated tumors receive the higher numerals.

- Grade I – Well differentiated squamous cell carcinoma
- Grade II – Moderately differentiated squamous cell carcinoma
- Grade III/IV – Poorly differentiated squamous cell carcinoma.

EXPRESSION OF SURVIVIN IN OSCC:

Lo Muzio et al (2001) in their case series identified that survivin was expressed in 64% of skin squamous cell carcinoma and 56% of oral squamous cell carcinomas. In contrast, normal skin epithelium, normal oral epithelium, and skin adnexa did not express survivin. They reported that survivin expression was significantly segregated with high-grade and undifferentiated tumours with size >1.5 cm and invariably associated with lymph node metastasis.[92]

Lo Muzio L (2003) reported positive survivin expression in 82.7% of cases of oral carcinoma and 100% of metastatic lesions. Conversely normal oral epithelium did not express survivin. There was also no significant correlation between survivin expression and age, sex, tumor size, the presence of lymph node and distant metastases. The authors stated that survivin expression was increased in poorly differentiated tumours, even if differences were not statistically significant.[1]

Lin CY et al (2005) investigated the immunohistochemical expression of survivin in oral epithelial dysplasia and OSCC. Cytoplasmic survivin staining was detected in 97% of oral epithelial dysplasia specimens and 98% of OSCC specimens but not in adjacent normal oral mucosal tissues. The labeling index for survivin significantly increased from ED to OSCC samples. They stated that their results indicated that survivin expression may be an important early event in oral carcinogenesis and predicts unfavorable prognosis for OSCC.[80]

Muzio et al (2005) analyzed OSCC tissues by immunohistochemistry for expression of survivin and found positivity in all the cases. Their results suggest that survivin expression may identify cases of OSCC with more aggressive and invasive phenotype and hence could influence the decision for the therapy at the time of diagnosis.[93]

Jane C et al (2006) analyzed the expression of apoptosis regulating genes such as survivin, Bcl-2, Bax and p53 in precancerous and cancerous lesions of the buccal mucosa of Indian tobacco chewers.Survivin, Bcl-2 and Bax expression was found to increase with increased grade of malignancy and was statistically most significant .The authors concluded that an increased expression of survivin in high-grade tumors suggests that the protein is likely to significantly contribute to apoptosis resistance in response to therapy.[94]

Jinbu Y et al (2006) reported an overall survivin positivity of 58% of OSCC in their study samples. The percentage of survivin-positive specimens in the T1+T2 group was significantly higher than that in the T3+T4 group and the percentage of survivin-positive specimens in the N0 group was also significantly higher than that in N+ group. They also noted a slightly higher percentage of survivin-positive specimens in the gingival cancer group compared with the tongue cancer group .They reached a conclusion that survivin was preferentially expressed in non-advanced, non-metastic, and chemotherapy-sensitive oral squamous cell carcinoma.[95]

Khan Z et al (2009) investigated the status of p53 in relation to survivin to determine the potential involvement in oral tumorigenesis and analysed the distribution of survivin in OSCC and oral premalignant lesions. Positive staining for

survivin was found in 72% OSCC and 44% premalignant lesions with no immunoreactions in the corresponding normal tissues. For p53, 59% OSCC, 38% premalignant lesions and 14% normal tissues were positive. It was observed that the number of survivin positive cells was significantly higher in the p53-positive group. In majority of cases survivin was localized in cytoplasm, whereas p53 was restricted to the nucleus. The survivin expression levels in both OSCC and premalignant lesions were significantly higher than in normal oral tissues. The authors concluded that overexpression of survivin and p53 in premalignant lesions suggested a role in early stages of oral carcinogenesis.[96]

Kim YH et al (2010) conducted a study for the assessment of the diagnostic and prognostic significance of survivin in a series of primary OSCC through immunohistochemistry. Survivin expression was detected in all cases at varying levels but was not observed in normal gingival keratinocyte cells. Clinicopathological analysis revealed a significant correlation between survivin expression and lymph node metastasis and proliferation.[97]

Freier K et al (2010) did a microarray analysis to study, whether increased copy numbers of the survivin-encoding gene BIRC5 results in elevated survivin levels and whether BIRC5 and survivin could serve as progression markers in the clinical course of OSCC, using immunohistochemnistry and fluorescence in situ hybridization. High survivin expression was found in 67.3% of cases to predict increased survival rate. They concluded that high survivin expression might be useful to identify OSCC patients, who would benefit from radio therapy.[98]

Su L et al (2010) evaluated the correlation between survivin mRNA expression and clinicopathologic features in OSCC. Survivin mRNA was assayed by reverse-transcription polymerase chain reaction assay and survivin protein by immunohistochemistry. The immunostaining of survivin protein was significantly stronger in OSCC tissues than in corresponding nontumor tissues. Moreover, high survivin mRNA expression was correlated with poor tumor differentiation and higher clinical stage. Authors concluded that siRNA-mediated survivin down-regulation could significantly inhibit proliferation and induce apoptosis and enhanced chemosensitivity of OSCC cells.[99]

Zhang et al (2013) studied the association of clinical parameters and prognosis with immunohistochemical expression of p21, p27 and survivin in OSCC. Their results revealed that p21 plays a predominant role in inhibiting apoptosis, likely through interactions with p27 and survivin and also that the expression of survivin was not a prognostic factor for OSCC.[100]

Lauxen et al (2014) investigated the expression of proteins involved in the development and maintenance of epithelia, cell cycle regulation, and apoptosis in OSCC samples. A total immunostaining score was calculated for survivin,bcl-2,epidermal growth factor receptor (EGFR), p21, p53, p63, and cleaved caspase-3. All cases showed > 75 % scores for p63, EGFR and survivin. The cells positive for cleaved caspase-3 and bcl-2 were less. The authors concluded that the high frequency of tumor cells expressing survivin and p63 highlights the role of these proteins in the malignant transformation of oral epithelium.[101]

MATERIALS & METHODS

The present study was carried out in the Department of Oral Pathology and Microbiology, Sree Mookambika Institute of Dental Sciences, Kulasekharam.

Study period: One and a half years

Study design: Retrospective study

Study subjects: Formalin fixed paraffin embedded blocks were obtained from the archives of Department of Oral Pathology an Microbiology, Sree Mookambika Institute of Dental Sciences, Kulasekharam. In addition samples were also obtained from the patients reporting to the OPD, Sree Mookambika Institute of Dental Sciences, Kulasekharam. Study group comprised of oral epithelial dysplasia (n=30) and OSCC (n=30).

Non-inflamed normal oral mucosa (n=30), obtained from adult patients undergoing extraction of third molar made up the control group. All biopsies were performed after obtaining an informed consent which was reviewed and approved by the institutional ethics committee of Sree Mookambika Institute of Dental Sciences, Kulasekharam. From each block, two sections were cut. The sections were standardized by maintaining the thickness at 3-4 microns. One set of sections were stained by Harris hematoxylin and eosin for histopathological diagnosis. The others were subjected for immunohistochemical staining for survivin.

Inclusion criteria:

Histopathologically confirmed cases of oral leukoplakia showing epithelial dysplasia and OSCC in the age group of 20-70 years were included in the study.

Exclusion criteria:

- ✓ Tissue sections without epithelium
- ✓ Non-oropharyngeal site

Inclusion criteria for normal tissue:

- ✓ Non- inflamed tissue from the buccal mucosa in relation to third molar in the age group of 20-70 years.

Exclusion criteria for normal tissue:

- ✓ Inflammatory conditions in relation to the third molar
- ✓ Patients with systemic diseases
- ✓ Smokers

Sample size:

Calculated based on the equation,

$$n = \frac{p1(100-p1)+ p2(100-p2) \text{ X } (Z\alpha+Z\beta)^2}{(p2-p1)^2}$$

Where p is the percentage of disease expressed oral epithelial dysplasia (n=30), OSCC (n=30) and normal tissue (n=30).

Samples were graded according to the following criteria:

Cases of oral leukoplakia were graded for dysplasia according to the WHO 2005 criteria. Oral squamous cell carcinoma cases were graded for differentiation according to the Broder's grading system.

WHO (2005) diagnostic criteria for dysplasia:

Dysplasia is a spectrum and no strict criteria exist to precisely divide this spectrum into mild, moderate and severe categories. When architectural disturbance is accompanied by cytologic atypia, the term dysplasia can be applied.[102]

Architectural changes:

- Irregular epithelial stratification
- Loss of polarity of basal cells
- Drop shaped rete ridges
- Abnormal superficial mitosis
- Premature keratinisation in single cells(dyskeratosis)
- Keratin pearls within the rete pegs
- Increased number and size of nucleoli

Cytological changes:

- Abnormal variation in nuclear size(anisonucleosis)
- Abnormal variation in nuclear shape(nuclear pleomorphism)

- Abnormal variation in cell shape (cellular pleomorphism)
- Increased nuclear-cytoplasmic ratio
- Increased nuclear size
- Atypical mitotic figures
- Increased number and size of nucleoli

Mild dysplasia

Architectural disturbance limited to the lower third of the epithelium accompanied by cytological atypia define the minimum criteria of dysplasia.

Moderate dysplasia

Architectural disturbance extending into the middle third of epithelium is the initial criterion to recognizing this category. However consideration of degree of cytologic atypia may require upgrading.

Severe dysplasia

Recognition of severe dysplasia starts with greater than two thirds of epithelium showing architectural disturbance with associated cytologic atypia. However architectural disturbance extending into middle third of epithelium with sufficient cytologic atypia is upgraded from moderate to severe dysplasia.

Criteria for Grading of Oral Squamous Cell Carcinoma:

The cases diagnosed histopathologically as oral squamous cell carcinoma were graded according to Broder's Grading System[102]

Grade1 (Well differentiated): 75% keratinocytes are well differentiated

Grade 2 (Moderately differentiated): >50% Keratinocytes are well differentiated

Grade 3 (Poorly differentiated): >25% Keratinocytes are well differentiated

Grade 4 (Anaplastic): <25% Keratinocytes are well differentiated

IMMUNOHISTOCHEMISTRY PROCEDURE:

Armamentarium:

1. Microtome (Spencers, Model no. 1010- SMT-006, India.)
2. Tissue floatation bath (Sh Brand, India)
3. Slide warmer (Sh Brand, India)
4. Refrigerator (Whirlpool 19 premier, India)
5. Digital weighing machine (INFRA. Digi, Model no. IN101L, India)
6. pH meter (ROY instruments Model no. IR-501, India)
7. Pressure cooker (IDEAL, India)
8. Electronic timer (PACER TM-103, India)
9. Light microscope (ADELTAPLAN2, Model no. AP40, ADELTA OPTEC, India)

10. APES (3-amino propyl triethoxysilane) coated slides (Pathnsitu Biotechnologies, India)
11. Beakers (Borosil, India)
12. Measuring jars (Borosil, India)
13. Coplin jars (Thermo scientific , USA)
14. Rectangular steel trays (Mopec, USA)
15. Glass rods (Borosil, India)
16. Slide carrier (Thermo scientific , USA)
17. Micropipettes (Accupipetes, India)
18. Cover-slips (Blue star, India)
19. Sterile gauze(Global Meditronic, India)
20. Marker pen (Camel, India)
21. Storage cabinet for archival blocks (Rescholar equipments, India)

Reagents for IHC

1. Conc. Hydrochloric acid (RANKEM, India)
2. TRIS and EDTA for TRIS EDTA Buffer (pH 8.5-9.5) (NICE, India)
3. TRIS and Sodium chloride for TRIS buffered saline-TBS (pH 7.4-7.6) (HPLC, India)
4. Deionized distilled water (NICE, India)
5. Mayer's Haematoxylin (HIMEDIA, India)

6. Alcohol 70%,80%,100% (CHANSHU YANGYVAN Chemicals, China)
7. Xylene (RANKEM, India)

Antibodies used:

- Primary antibody

  Survivin-EP119 Rabbit Monoclonal Antibody (PathnSitu, India)

- Secondary antibody

  Horse radish peroxidase (HRP) (ThermoScientific, USA)

Thermo Scientific secondary kit contains:

- ✓ Hydrogen peroxide block
- ✓ Ultra violet block
- ✓ Horse radish peroxidase(HRP)
- ✓ Chromogen DAB (3-Diaminobenzidine Tetrahydrochloride)

Procedure immunohistochemical staining

Preparation of sections:

Tissue sections of four micron thickness were made in a rotary semiautomatic microtome. The ribbons of tissue section were transferred onto the APES coated slide from the tissue floatation bath such that two tissue bits come on to the slide with a gap in between. One of the tissue sections was labeled positive and the other negative. In addition tissue section of normal stomach mucosa was kept as control.

Deparaffinization:

The slides with tissue sections were treated with two changes of xylene, 10 minutes each to remove paraffin wax. They were put in two changes of absolute alcohol followed by descending grades of alcohol (80% and 70%), for 5 minutes each for rehydration. Then they were transferred to distilled water for 5 minutes.

Antigen retrieval :

Preparation of antigen retrieval buffer:-

TRIS- EDTA buffer was prepared by dissolving 6.05g of TRIS and 0.75 g of EDTA in 1000ml of distilled water. The pH was adjusted between 8.5-9.5 using concentrated hydrochloric acid**.**

Method:-

The slides were transferred to TRIS- EDTA buffer in a coplin jar and heated in a pressure cooker for 5 minutes. After releasing the pressure, the pressure cooker was opened and slides were allowed to cool.

Preparation of TRIS buffered saline (Wash buffer):-

TRIS buffered saline was prepared by dissolving 0.605g of TRIS and 8.0 g of sodium chloride in 1000ml of distilled water. The pH was adjusted between 7.4-7.6 using concentrated hydrochloric acid**.** Then the slides were placed on a slide staining rack kept on a rectangular steel tray and washed in three changes TRIS buffered saline solution. Then the sections were blotted carefully with tissue paper.

Blocking endogenous peroxidase activity:

Slides were treated with hydrogen peroxide block for 10 minutes to quench endogenous peroxidase activity of cells that would otherwise result in non-specific staining. This was done by adding one drop of the hydrogen peroxide block from the Thermo secondary kit onto the tissue sections. Then the slides were washed in TRIS buffered saline solution for three changes and blotted dry.

Treatment with Ultra violet block reagent:

One drop of Ultra violet block reagent from the Thermo secondary kit was added to the sections and kept for 5 minutes. The slides were then wiped carefully with tissue paper without touching the tissue section to remove excess reagent.

Incubation with primary antibody:

Circles were drawn around the tissues, so that the antibodies added later on do not spread and are restricted to the circle. The primary antibody (Survivin) was added only to positive tissue on the slide and TRIS buffer is added to the negative tissue to prevent drying. The slides were incubated at room temperature for 1 hour. Then the sections were washed in three changes of TRIS buffered saline for 3 minutes each to remove the excess antibody. Then the slides were wiped carefully without touching the tissue section to remove excess TRIS buffered saline.

Incubation with secondary antibody:

A drop of HRP (secondary antibody) was added on both the sections and the slides were incubated for 30 minutes. The sections were washed in 3 changes of TRIS

buffered saline for 3 minutes each. The slides were wiped carefully without touching the tissue section to remove excess buffer.

Incubation with chromogen:

A drop of freshly prepared DAB (3' Diaminobenzidine Tetrahydrochloride - a substrate chromogen) was added on both sections and kept for 15-30 minutes. Slides were washed in TRIS buffered saline to remove excess DAB.

Counterstaining with hematoxylin:

Slides were counterstained with hematoxylin. Then the slides were placed in a tray of tap water for 5 minutes for the process of bluing.

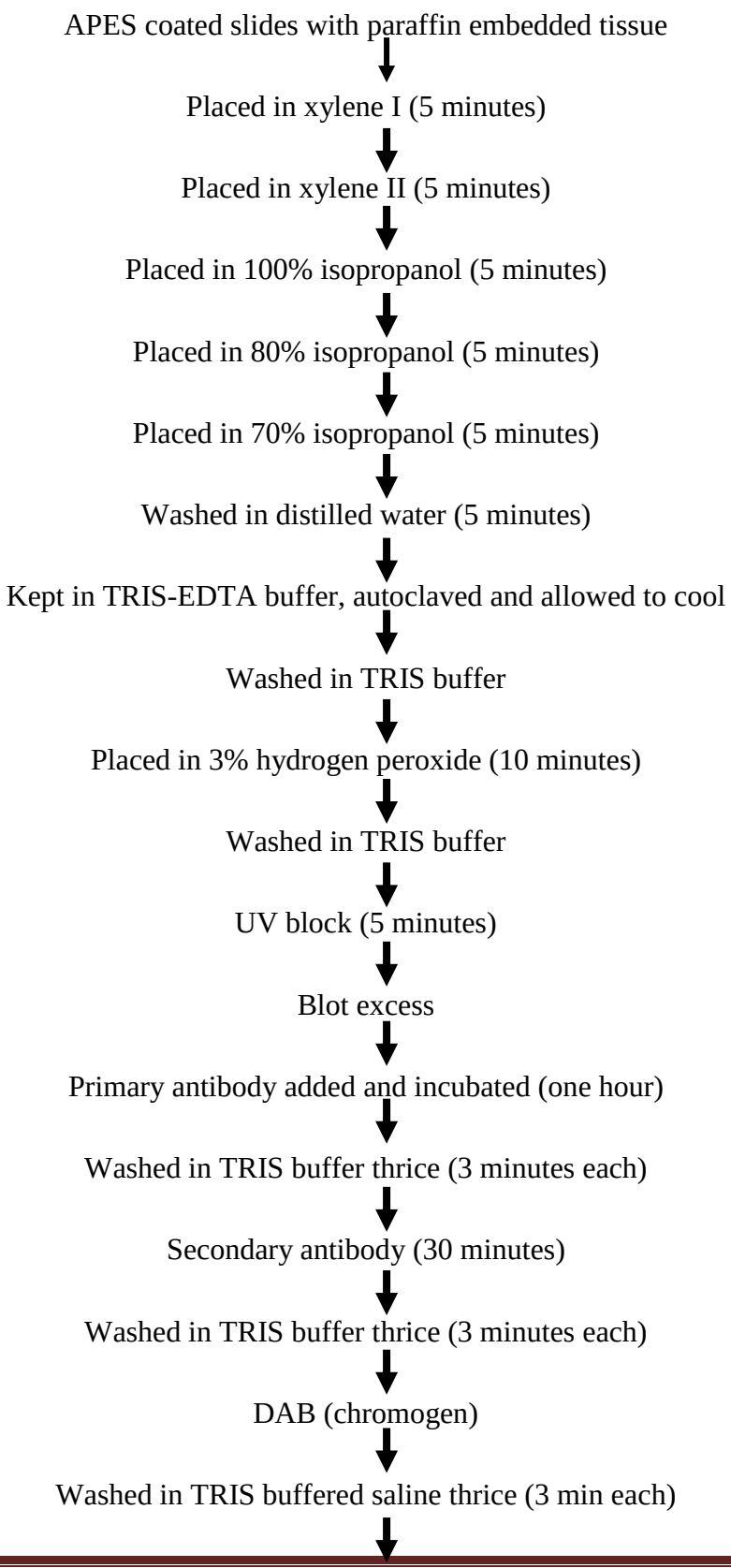
Dehydration:

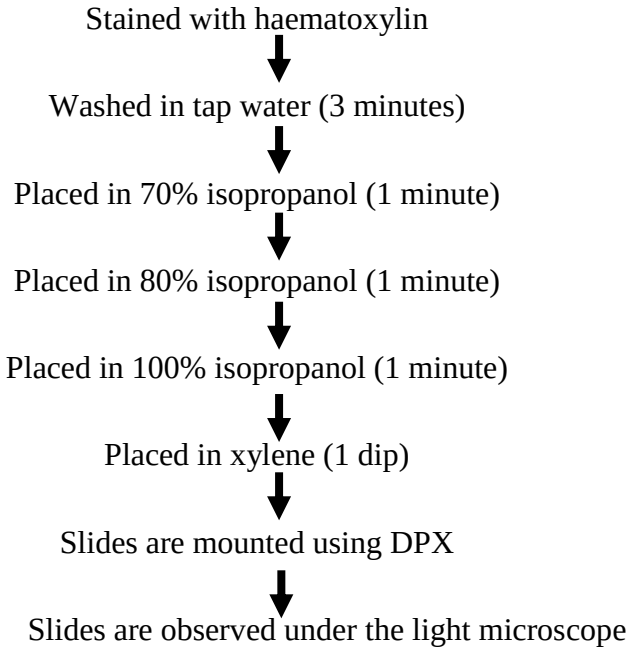
The slides were transferred to 70% alcohol, 80% alcohol, 100% alcohol and xylene.

Mounting:

The tissue sections were mounted with DPX.

IHC procedure flow chart:





Evaluation:

Slides were observed under light microscope and survivin expression in the cytoplasm of epithelial cells was graded according to the grading criteria followed by Yoshinori Jinbu et al (2006).[95] Grading was done by two oral pathologists to whom the clinical data was unknown. Expression of survivin in normal stomach mucosa served as the positive control.

All of the cases were graded into three groups based on the degree of survivin staining:

- Grade 0 : Negative expression
- Grade 1+ : Weak expression
- Grade 2+ : Strong expression

Statistical analysis:

Data entry done on data entry sheet and later transferred to microsoft excel sheet. Data was analysed using computer software SPSS (Statistical Package for Social Sciences) version16 for windows operating system.

COLOUR PLATES

CP-1: Storage cabinet for archival blocks

CP-2: Semiautomatic microtome

CP-3: Tissue floatation bath

CP-4: Slide warming table

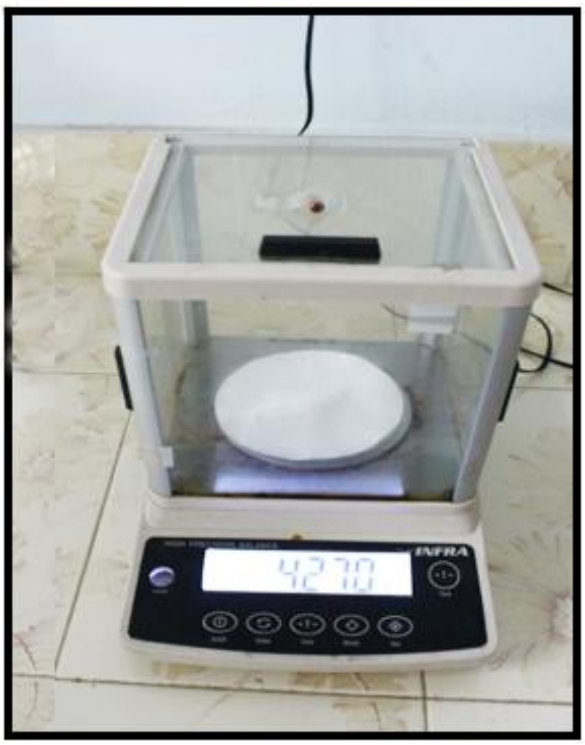
CP-5: Armamentarium for the preparation of buffer solutions

CP-6 :Pressure cooker for antigen retrieval

CP-7 : Reagents used for immunohistochemical staining

CP-8 : pH meter

CP-9 : Digital weighing machine



CP-10 : Survivin – Primary Antibody

RESULTS & OBSERVATIONS

The present study was carried out in department of oral pathology and microbiology, Sree Mookambika Institute of Dental Sciences, Kulasekharam. 30 cases of normal buccal mucosa constituted the control group whereas 30 cases of oral epithelial dysplasia (OED) & 30 cases of squamous cell carcinoma (OSCC) constituted the study group.

Based on clinical and histopathological correlation the 30 cases of OED were further divided into mild, moderate & severe where as OSCC cases were further graded into well differentiated and moderately differentiated. Poorly differentiated OSCC cases were not registered during the course of the study and few samples in departmental archives were disputable and hence, were not considered in the present study.

In the present study the patients with oral epithelial dysplasia were in the age group of 20 – 70 years with mean age of 54.2 years and there was male predominance with male to female ratio of 3:1. The patients with oral squamous cell carcinoma in our study were in the age group of the 49 – 74 years with the mean age of 59.4 years and there was equal occurrence among both sexes with the male to female ratio of 1:1.

Documentation of the associated habits & common site of the lesion showed betelnut chewing as the most prevalent cause for both epithelial dysplasia and squamous cell carcinoma whereas buccal mucosa was the most favorable site of occurrence in both the cases.

Expression of survivin was analysed using standard immunohistochemical procedure. The intensity of survivin expression in the cytoplasm of epithelial cells

was graded according to the grading criteria followed by Yoshinori Jinbu et al (2006). All the values were expressed in terms of number and percentage (%). The results obtained were tabulated and subjected to statistical analysis.

IMMUNOHISTOCHEMICAL EXPRESSION OF SURVIVIN IN ORAL EPITHELIAL DYSPLASIA

Table – 1: Distribution of samples according to selected clinicopathological parameters in oral epithelial dysplasia

AGE (Range: 20 – 70 years, Mean: 54.2)		Number	Percentage (%)
SEX	Male	22	73.3
	Female	8	26.7
SITE	Tongue	5	16.7
	Buccal mucosa	20	66.7
	Lip	5	16.7
HISTOLOGICAL GRADES OF DYSPLASIA	Mild dysplasia	7	23.3
	Moderate dysplasia	10	33.3
	Severe dysplasia	13	43.3

Table – 2: Association between clinicopathological parameters and grades of survivin expression in oral epithelial dysplasia. (% in brackets)

PARAMETER		Grade 0	Grade 1	Grade 2	P value
SEX	Male	1 (4.5)	17 (77.3)	4 (18.2)	0.344
	Female	1(12.5)	7 (87.5)	0(0)	
SITE	Tongue	0 (0)	4 (80)	1(20)	0.524
	Buccal mucosa	2 (10)	16 (80)	2(10)	
	Lip	0(0)	4(80)	1 (20)	
HISTOLOGICAL GRADES OF DYSPLASIA	Mild dysplasia	1(14.3)	5 (71.4)	1 (14.3)	0.667
	Moderate dysplasia	1(10)	8(80)	1(10)	
	Severe dysplasia	0(0)	11(84.6)	2(15.4)	

IMMUNOHISTOCHEMICAL EXPRESSION OF SURVIVIN IN ORAL SQUAMOUS CELL CARCINOMA

Table – 3: Distribution of samples according to selected clinicopathological parameters in oral squamous cell carcinoma.

AGE (Range: 49 – 74 years, Mean: 59.4)		Number	Percentage (%)
SEX	Male	13	43.3
	Female	17	56.7
SITE	Tongue	4	13.3
	Buccal mucosa	21	70.0
	Lip	2	6.7
	Palate	3	10.0
HISTOLOGICAL GRADES OF DIFFERENTIATION	Well differentiated	15	50.0
	Moderately differentiated	15	50.0

Table – 4: Association between clinicopathological parameters and grades of survivin Expression in oral squamous cell carcinoma. (% in brackets)

PARAMETER		Grade 1	Grade 2	P value
SEX	Male	6 (46.2)	7(53.8)	0.183
	Female	12 (70.6)	5(29.4)	
SITE	Tongue	2(50)	2 (50)	0.666
	Buccal mucosa	12(57.1)	9(42.9)	
	Lip	2(100)	0 (0)	
	Palate	2 (66.7)	1(33.3)	
HISTOLOGICAL GRADES OF DIFFERENTIATION	Well differentiated	6 (40)	9 (60)	0.028
	Moderately differentiated	12 (80)*	3 (20)*	

(*P<0.05 significant compared well differentiated with moderately differentiated)

IMMUNOHISTOCHEMICAL EXPRESSION OF SURVIVIN IN NORMAL MUCOSA

Table – 5: Distribution of sample according to selected clinical parameters in normal mucosa

AGE (Range:19 – 43, Mean:26.4)		Number	Percentage (%)
SEX	Male	16	53.3
	Female	14	46.7
SITE	Buccal mucosa	16	53.3
	Gingiva	14	46.7

Table – 6: Association between clinical parameters and grades of survivin expression in normal mucosa. (% in brackets)

PARAMETER		Grade 0	Grade 1	Grade 2	P value
SEX	Male	13 (81.3)	3 (18.8)	0(0)	0.822
	Female	12(85.7)	1(7.1)	1(7.1)	
SITE	Gingiva	11 (78.6)	2 (14.3)	1(7.1)	0.137
	Buccal mucosa	14(87.5)	2(12.5)	0(0)	

(*P<0.05 significant compared between males and females, P<0.05 significant compared gingiva with buccal mucosa)

COMPARSION OF SURVIVIN EXPERSSION IN ORAL SQUAMOUS CELL CARCINOMA, ORAL EPITHELIAL DYSPLASIA AND NORMAL MUCOSA

Table – 7: Comparison of survivin expression in oral epithelial dysplasia, oral squamous cell carcinoma and normal mucosa.

Grade	Oral epithelial dysplasia	Oral squamous cell carcinoma	Normal mucosa	P value
Grade 0	2 (6.7)	0(0)	25 (83.3)*,#	0.001
Grade 1	24 (80)	18(60)	4(13.3)*,#	
Grade 2	4(13.3)	12(40)	1(3.3)#	

(*P<0.05 significant compared Oral epithelial dysplasia with normal mucosa,

#P<0.05 significant compared Oral squamous cell carcinoma with oral epithelial dysplasia)

PAIR WISE COMPARISON OF SURVIVIN EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA AND ORAL EPITHELIAL DYSPLASIA WITH THAT IN NORMAL MUCOSA

Table – 8: Comparison of survivin expression in oral squamous cell carcinoma and oral epithelial dysplasia with that in normal mucosa.

Survivin expression	Oral epithelial dysplasia	Oral squamous cell carcinoma	Normal mucosa	P value
Negative Expression (Not expressed or Grade-0)	2	0	25*	0.001
Positive expression (Expressed or Grade-1 & 2)	28$^{\#}$	30$^{\#}$	5$^{*,\#}$	
P value	0.01	0.001	0.01	

(*P<0.05 significant compared survivin expression between the groups, $^{\#}$P<0.05 significant compared survivin expression within the groups. (n=30))

PAIR WISE COMPARISON OF SURVIVIN EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA AND ORAL EPITHELIAL DYSPLASIA WITH THAT IN NORMAL MUCOSA

Table – 9: Comparison of survivin expression in oral squamous cell carcinoma and oral epithelial dysplasia with that in normal mucosa. (In %)

Survivin expression	Oral epithelial dysplasia	Oral squamous cell carcinoma	Normal mucosa	P value
Negative Expression (Not expressed or Grade-0)	6.67 (2)	0(0)	83.33(25)*	0.001
Positive expression (Expressed or Grade-1 & 2)	93.33(28)$^{\#}$	100(30)$^{\#}$	16.67 (5)*,$^{\#}$	
P value	0.01	0.001	0.01	

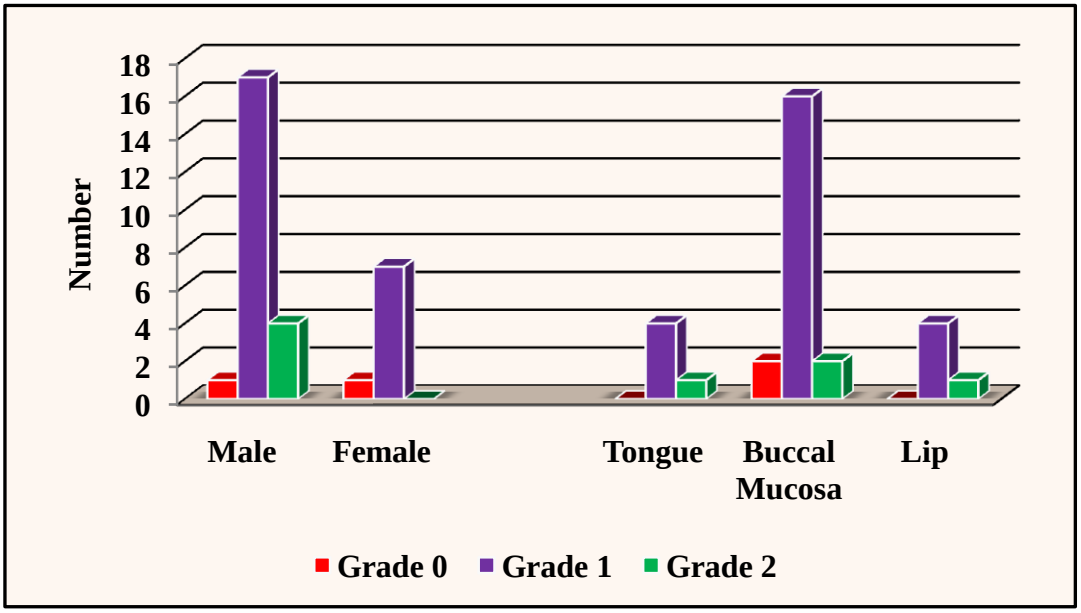
(*P<0.05 significant compared oral epithelial dysplasia with others, $^{\#}$P<0.05 significant compared oral squamous cell carcinoma with normal mucosa)

Table – 10: Comparison of total, grade-1 and grade-2 survivin expression in oral epithelial dysplasia & oral squamous cell carcinoma. (in %)

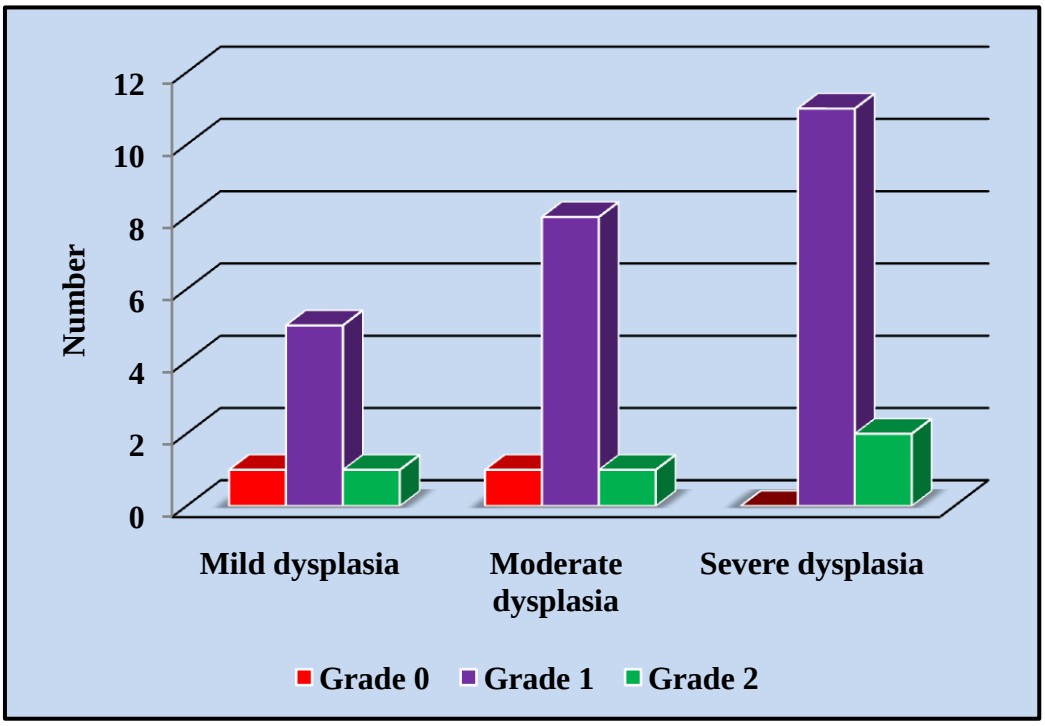
GRADE OF SURVIVIN EXPRESSION	ORAL EPITHELIAL DYSPLASIA		ORAL SQUAMOUS CELL CARCINOMA	
	Number	%	Number	%
Grade 1	24	80.00	18	60.00
Grade 2	4	13.30	12	40.00
Total expression	28	93.30	30	100.00

GRAPHS

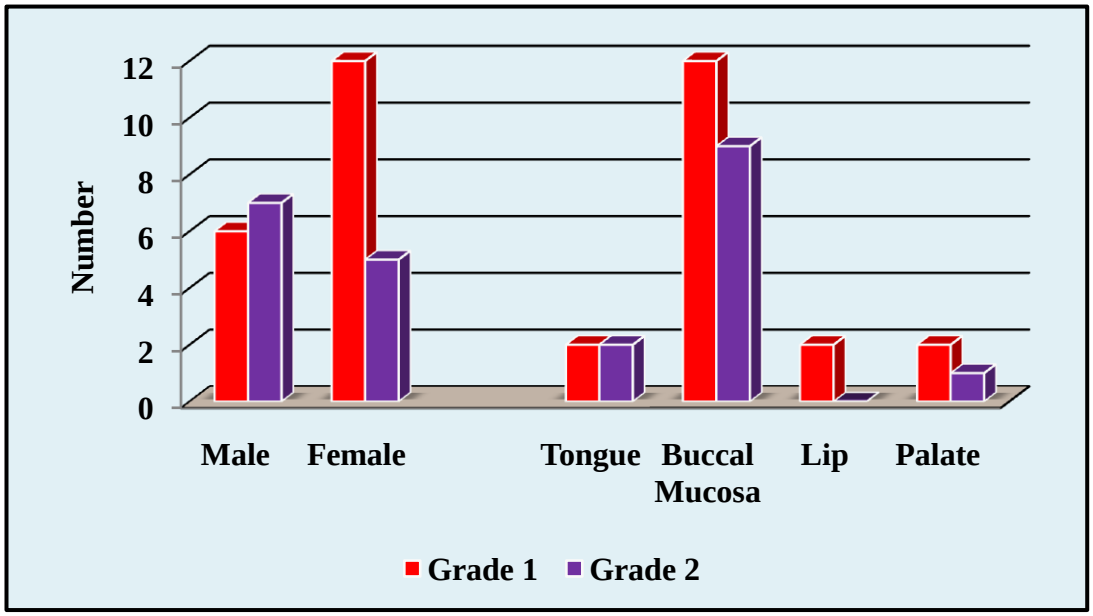
Graph – 1: Association between gender and site to grades of survivin expression in oral epithelial dysplasia



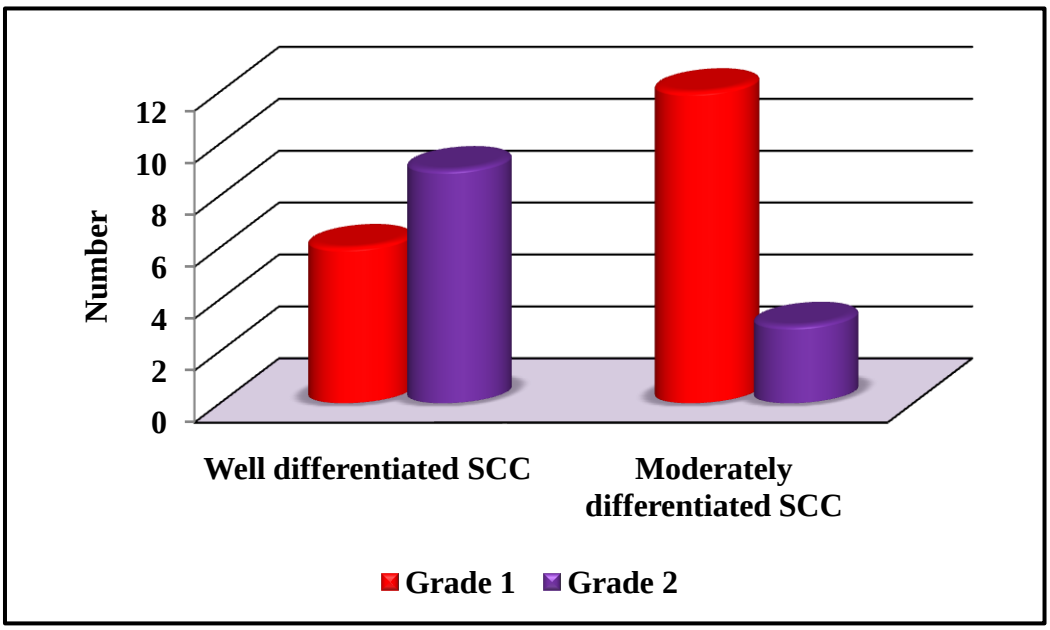
Graph – 2: Association between clinicopathological parameters and grades of survivin expression in oral epithelial dysplasia



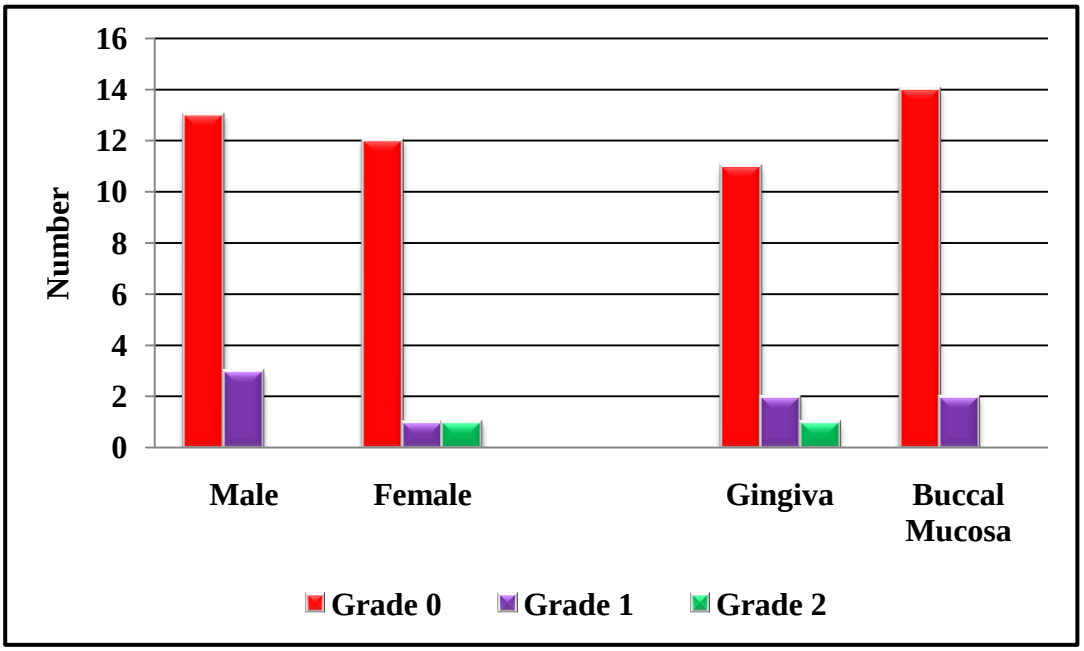
Graph – 3: Association between gender and site parameters to grades of survivin expression in oral squamous cell carcinoma



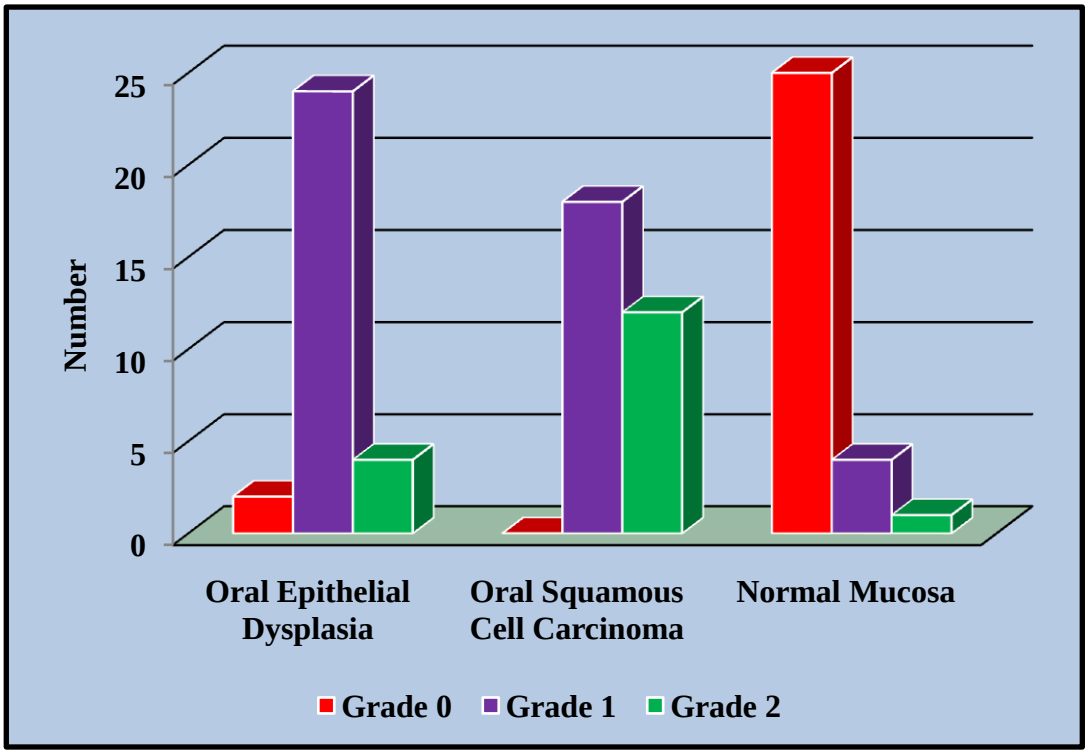
Graph – 4: Association between clinicopathological parameters and grades of survivin expression in oral squamous cell carcinoma



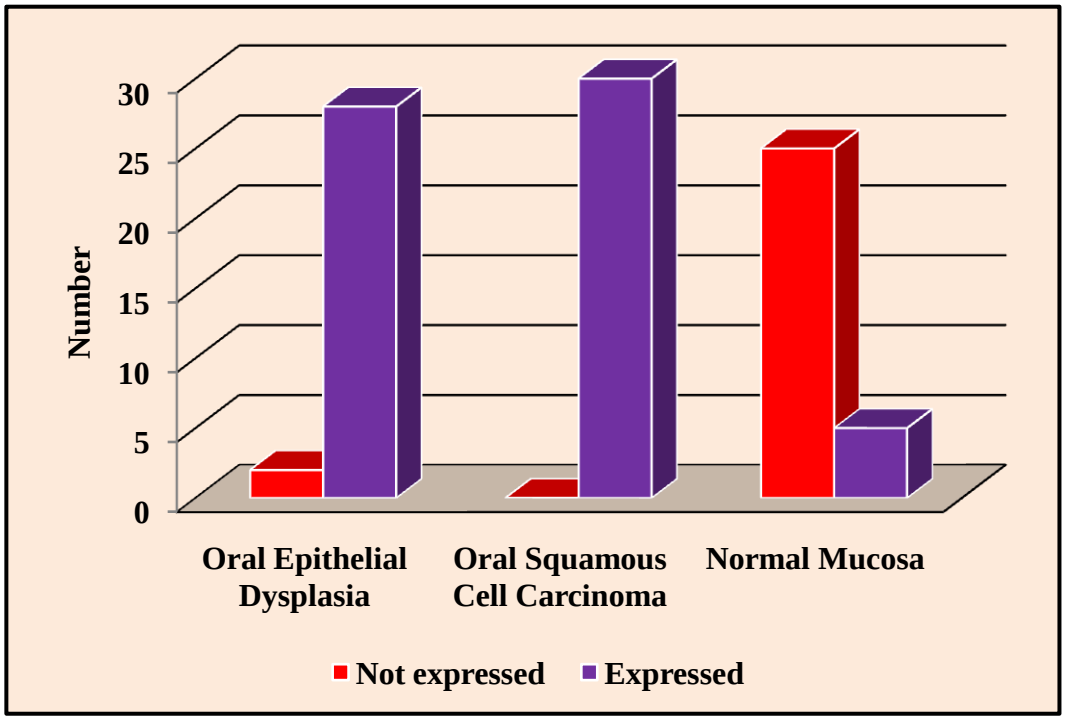
Graph – 5: Association between clinical parameters and grades of survivin expression in normal mucosa



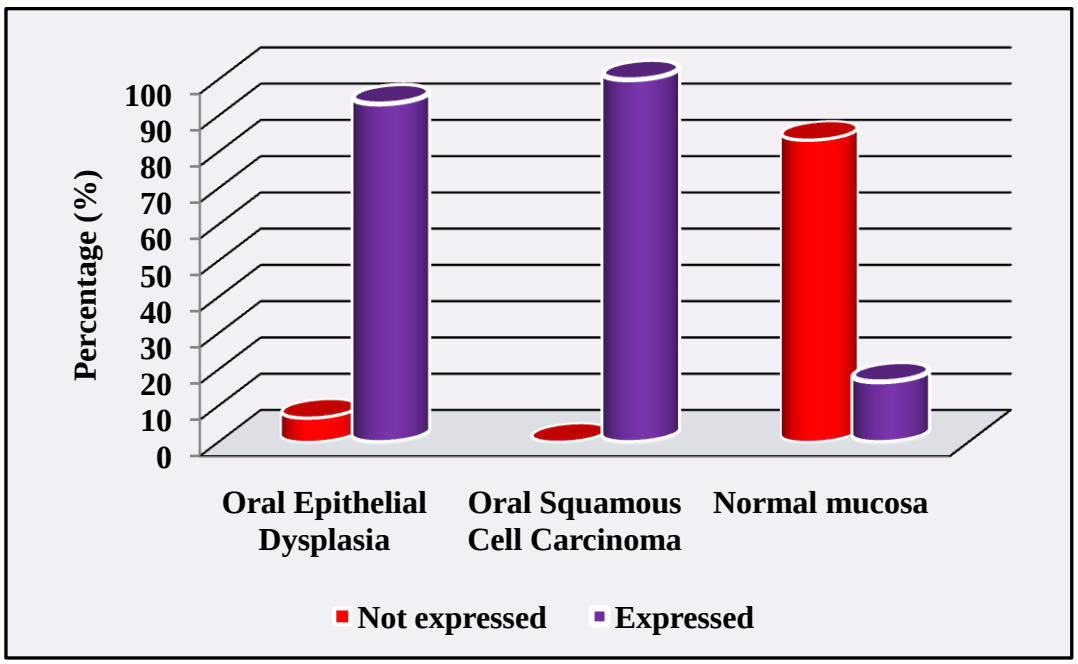
Graph – 6: Comparison of grades of survivin expression in oral epithelial dysplasia, oral squamous cell carcinoma and normal mucosa.



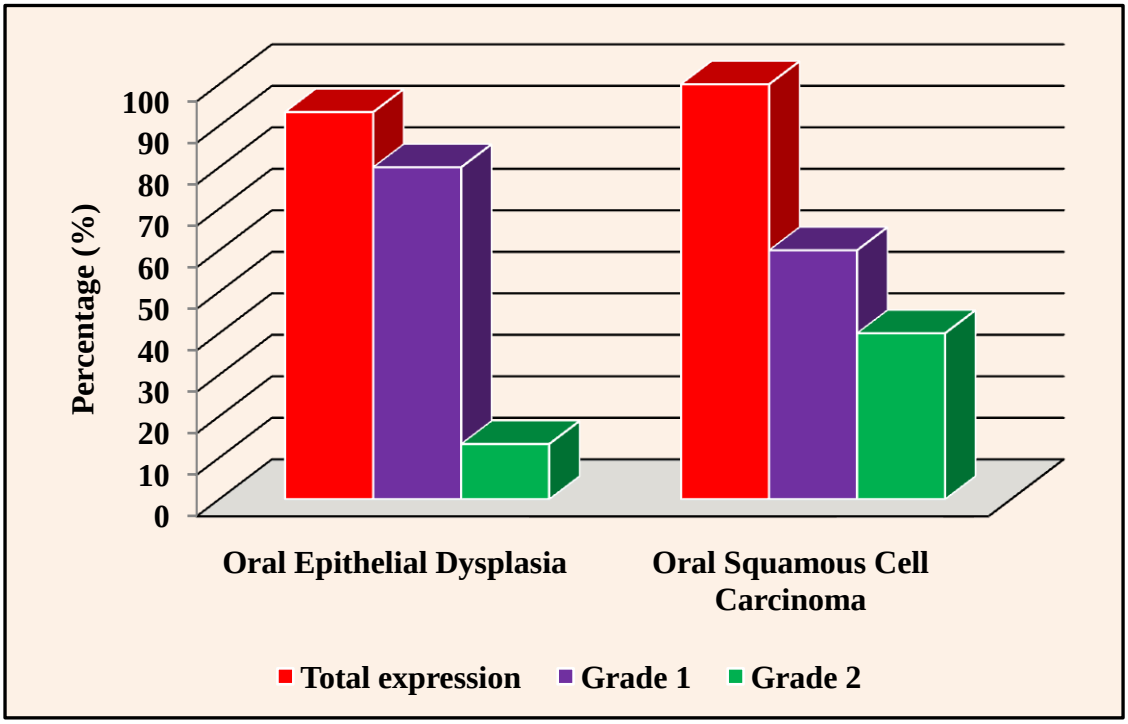
Graph – 7: Comparison of survivin expression in oral epithelial dysplasia and oral squamous cell carcinoma with that in normal mucosa.



Graph – 8: Comparison of survivin expression in oral epithelial dysplasia, oral squamous cell carcinoma and normal mucosa.

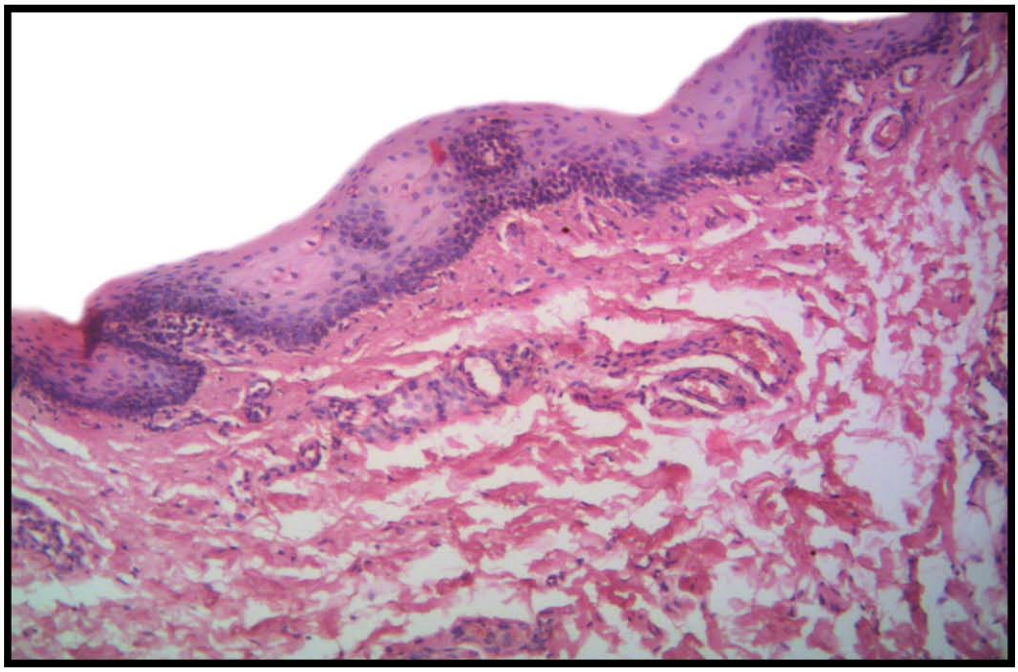


Graph – 9: Comparison of total, grade-1 and grade-2 survivin expression in oral epithelial dysplasia & oral squamous cell carcinoma. (in %)

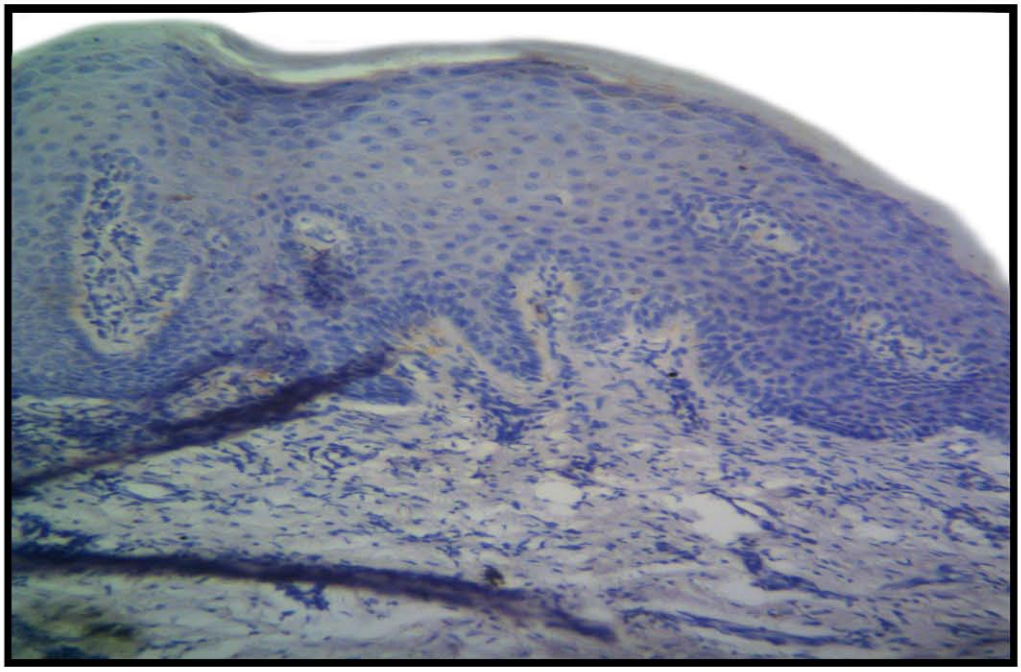


COLOUR PLATES

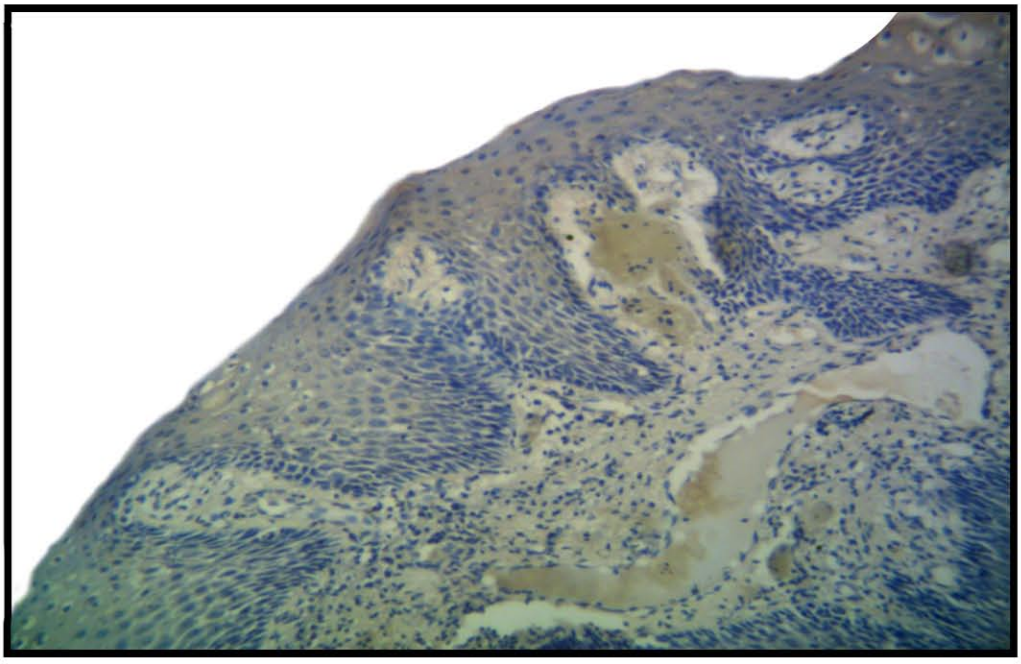
CP 11 :Photomicrograph showing Normal mucosa [H&E stained] (10x)



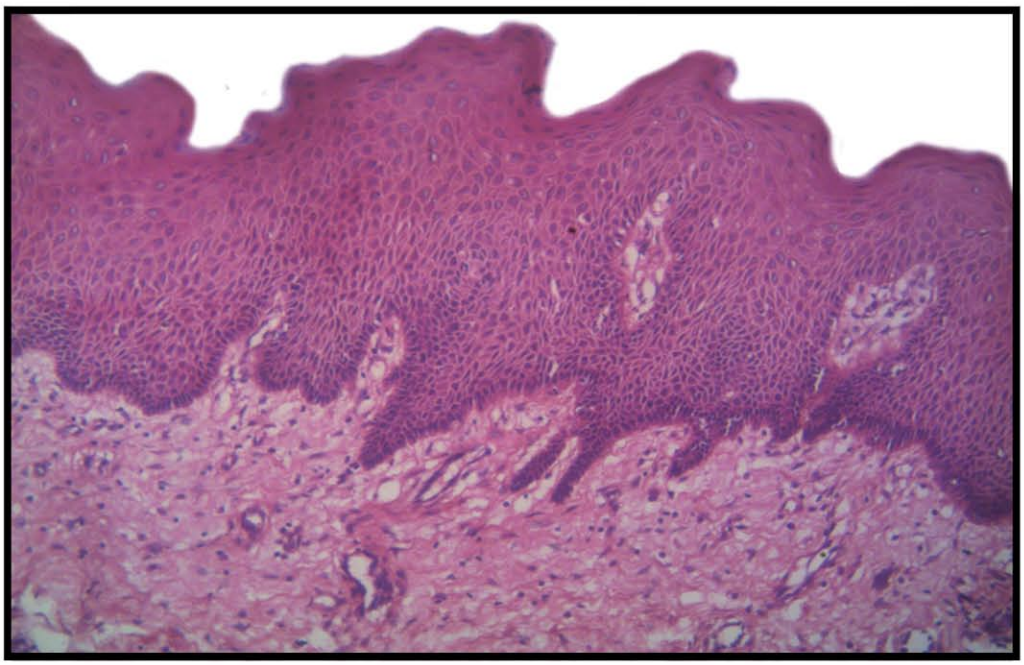
CP 12: Photomicrograph showing negative survivin expression on the normal mucosa (10x)



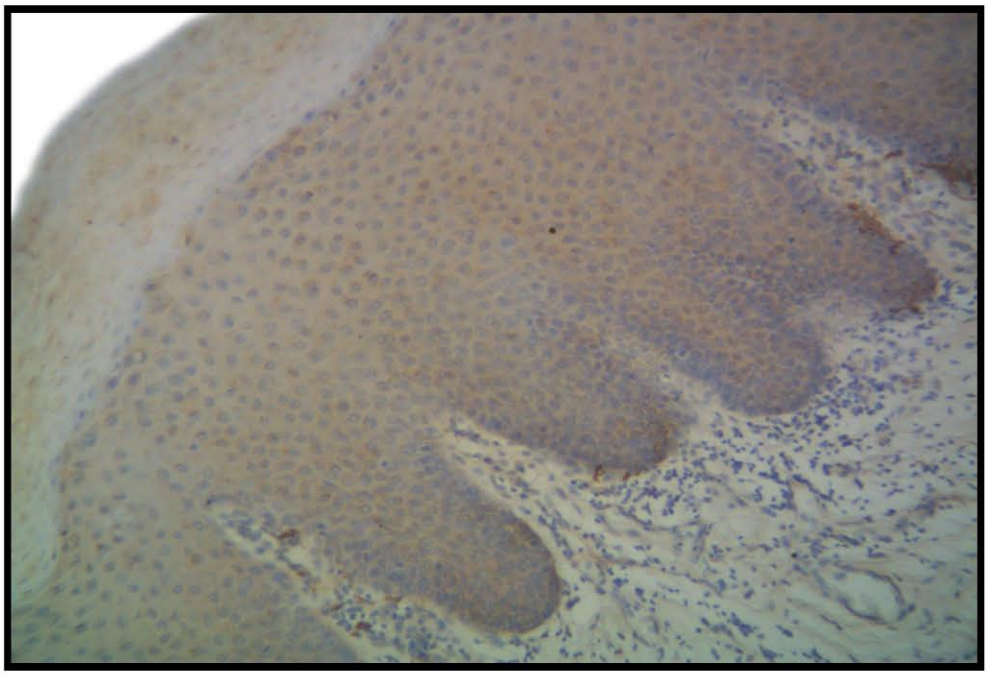
CP 13: Photomicrograph showing survivin expression of Grade 1+ on the normal mucosa (10x)



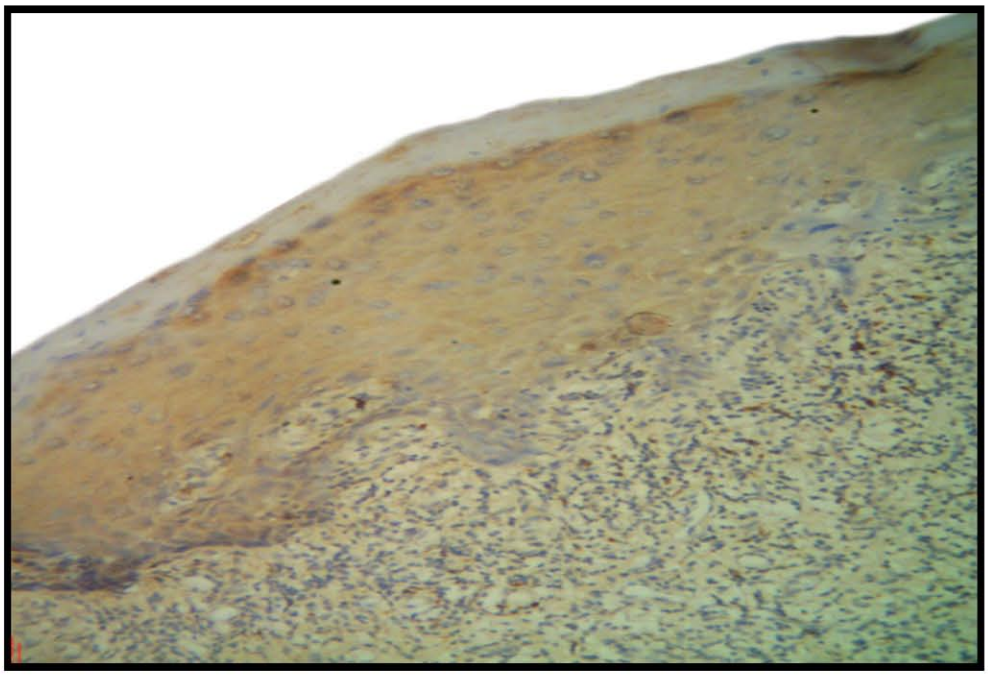
CP 14: Photomicrograph showing Mild dysplasia [H&E stained] (10x)



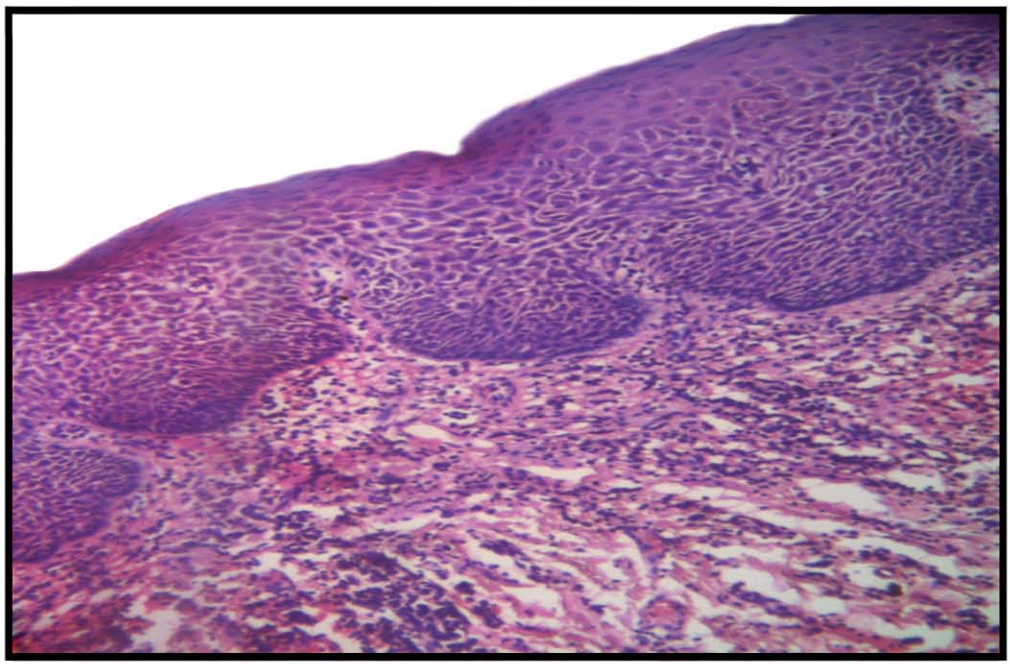
CP 15: Photomicrograph showing survivin expression of Grade 1+ on Mild dysplasia (10x)



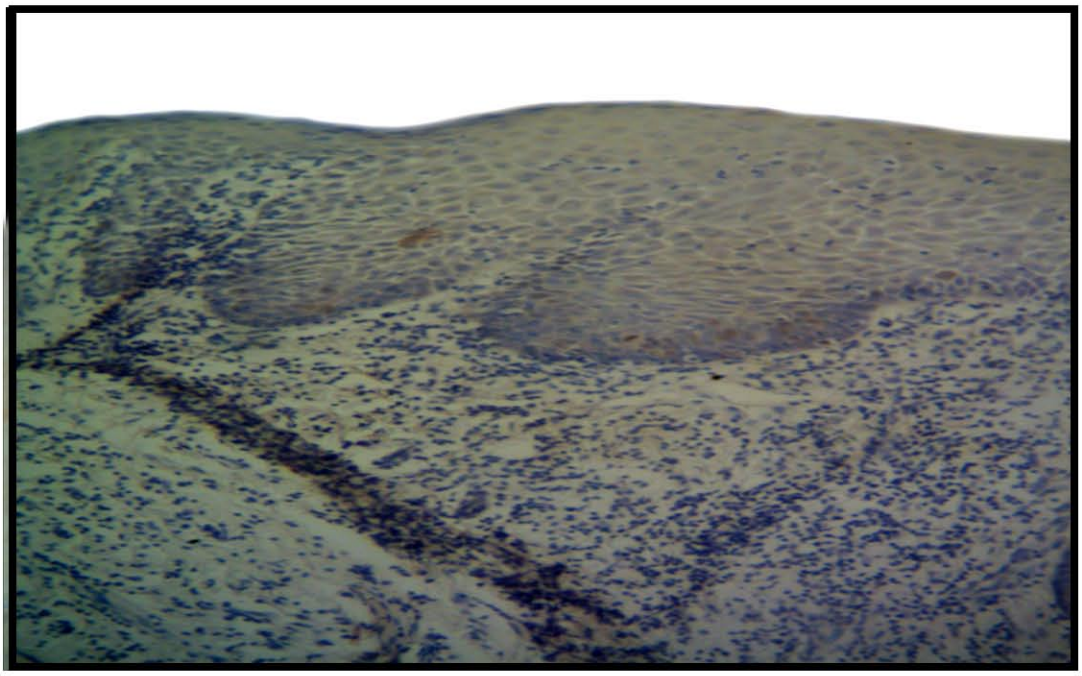
CP 16: Photomicrograph showing survivin expression of Grade 2+ on Mild dysplasia (10x)



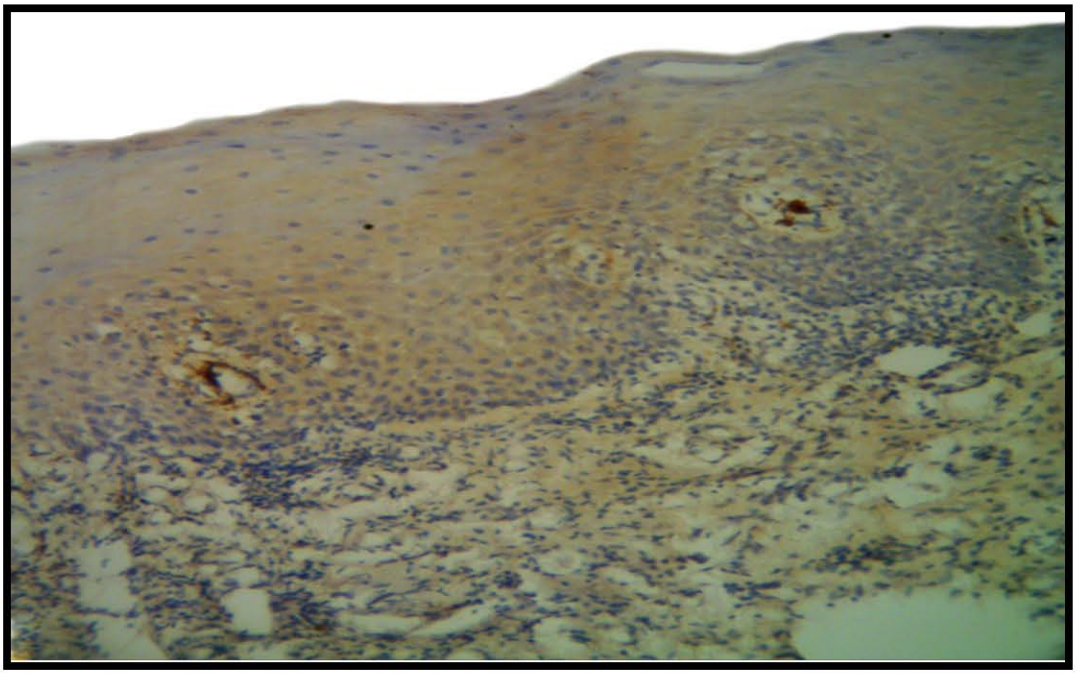
CP 17: Photomicrograph showing Moderate dysplasia [H&E stained] (10x)



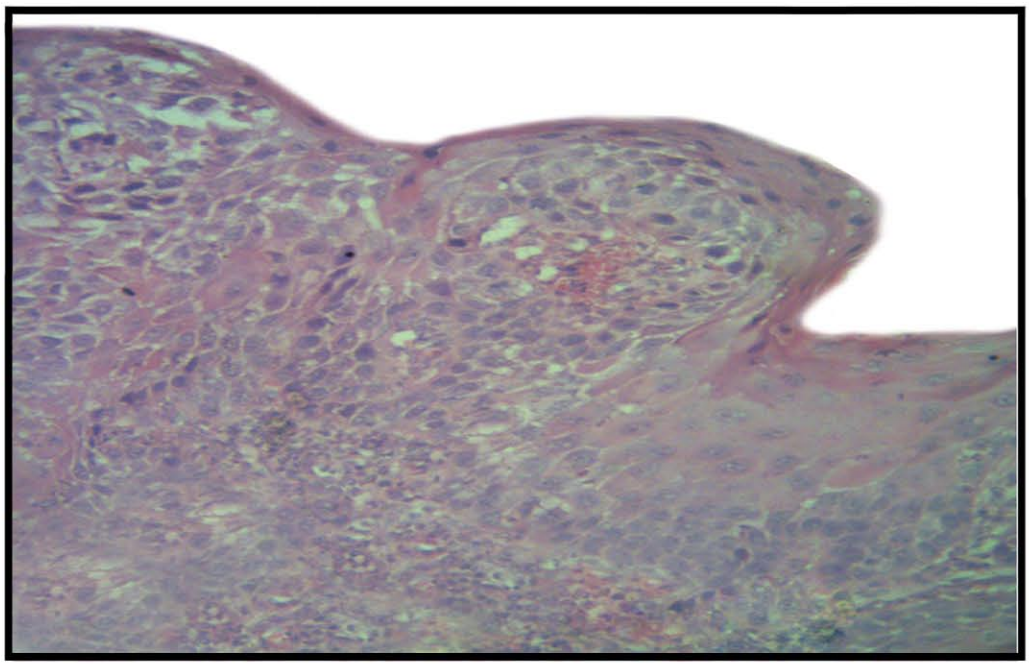
CP 18 : Photomicrograph showing survivin expression of Grade 1+ on Moderate dysplasia (10x)



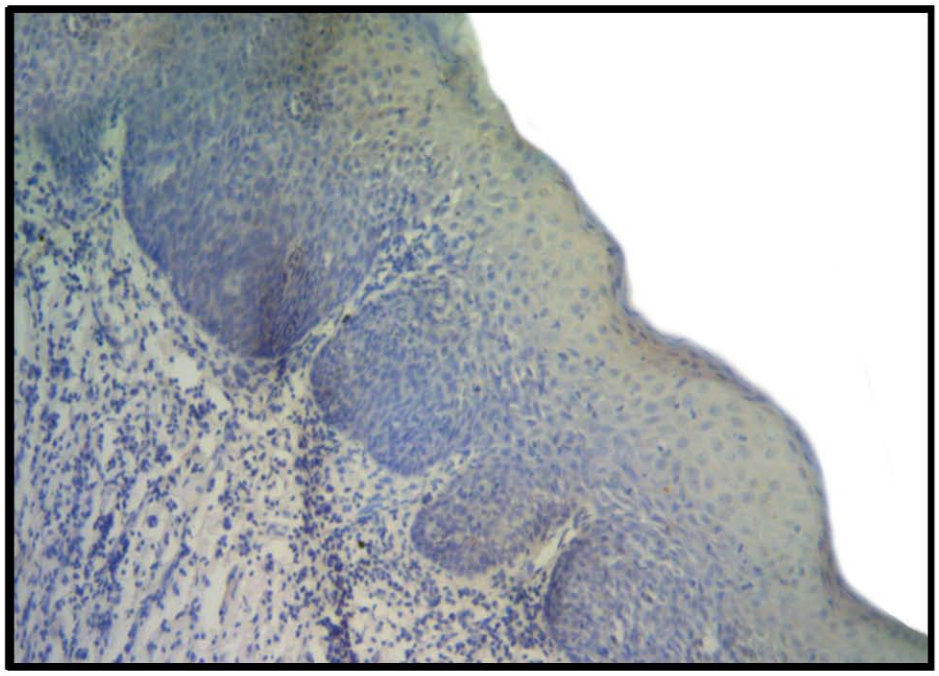
CP 19 : Photomicrograph showing survivin expression of Grade 2+ on Moderate dysplasia (10x)



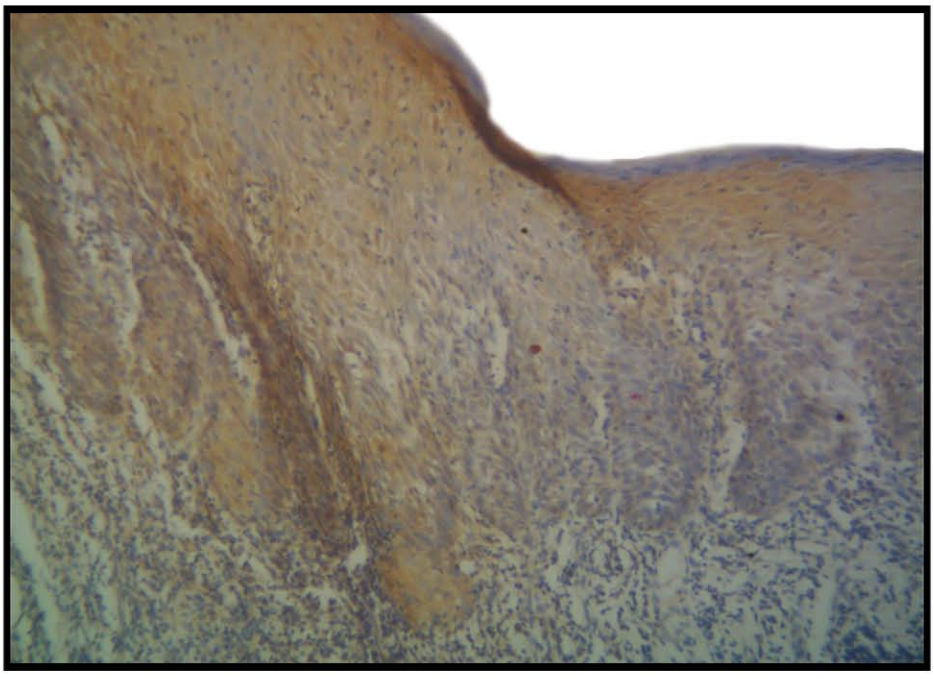
CP 20: Photomicrograph showing Severe dysplasia [H&E stained] (10x)



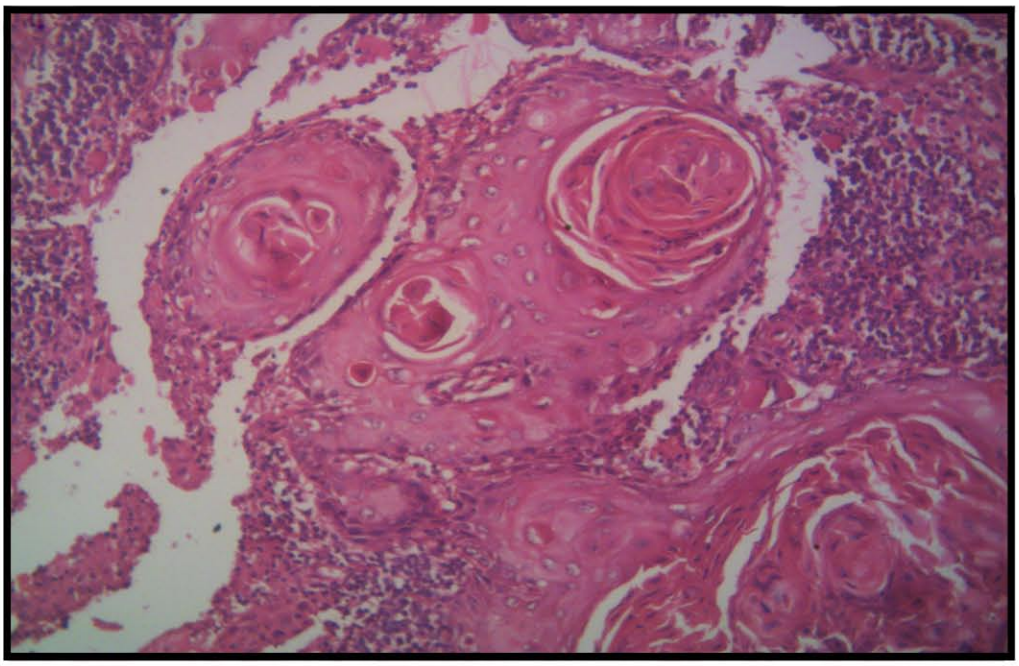
CP 21 : Photomicrograph showing survivin expression of Grade 1+ on Severe dysplasia (10x)



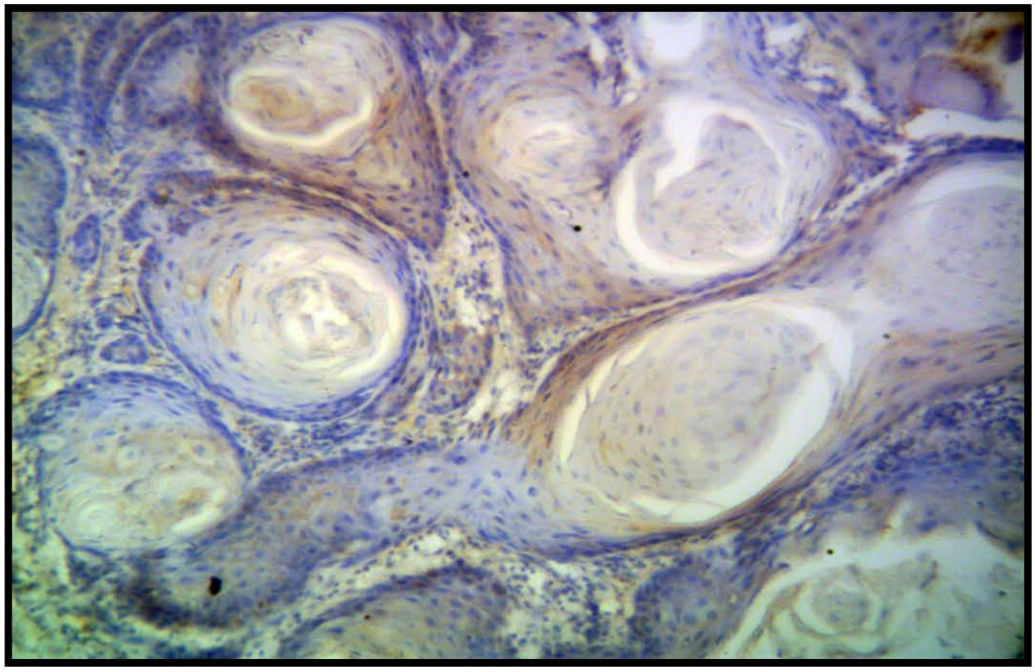
CP 22: Photomicrograph showing survivin expression of Grade 2+ on Severe dysplasia (10x)



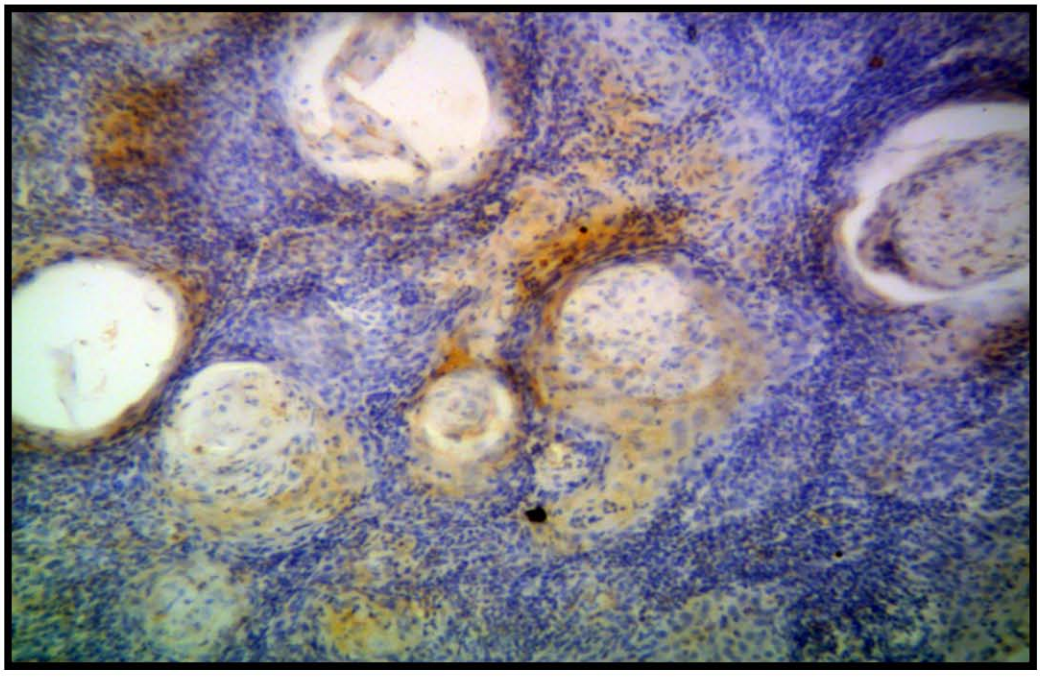
CP 23: Photomicrograph showing Well differentiated squamous cell carcinoma [H&E stained] (10x)



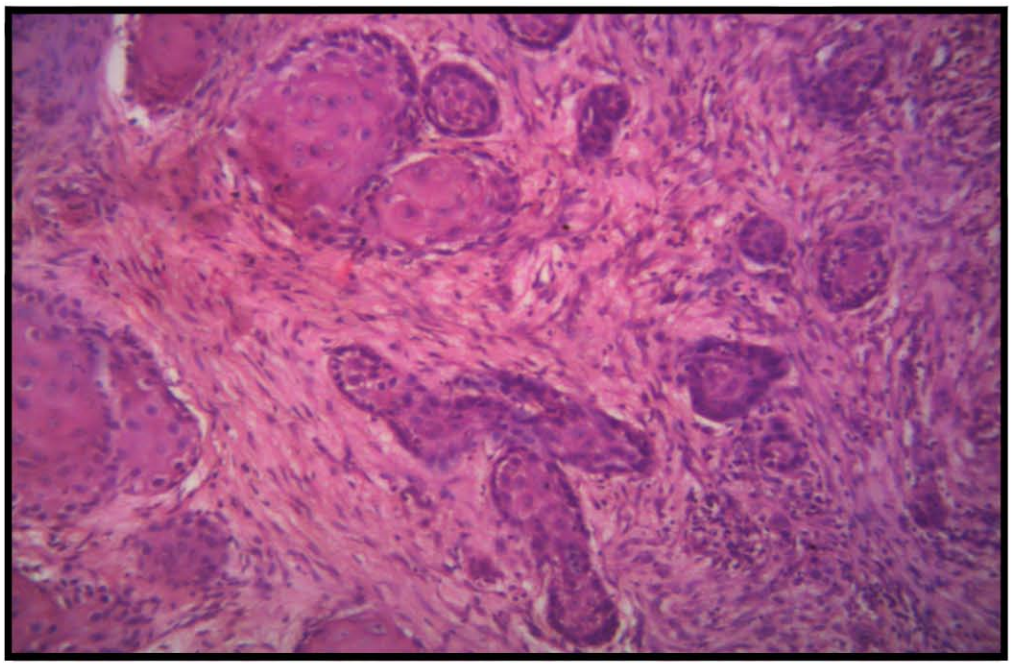
CP 24: Photomicrograph showing survivin expression of Grade 1+ on Well differentiated squamous cell carcinoma (10x)



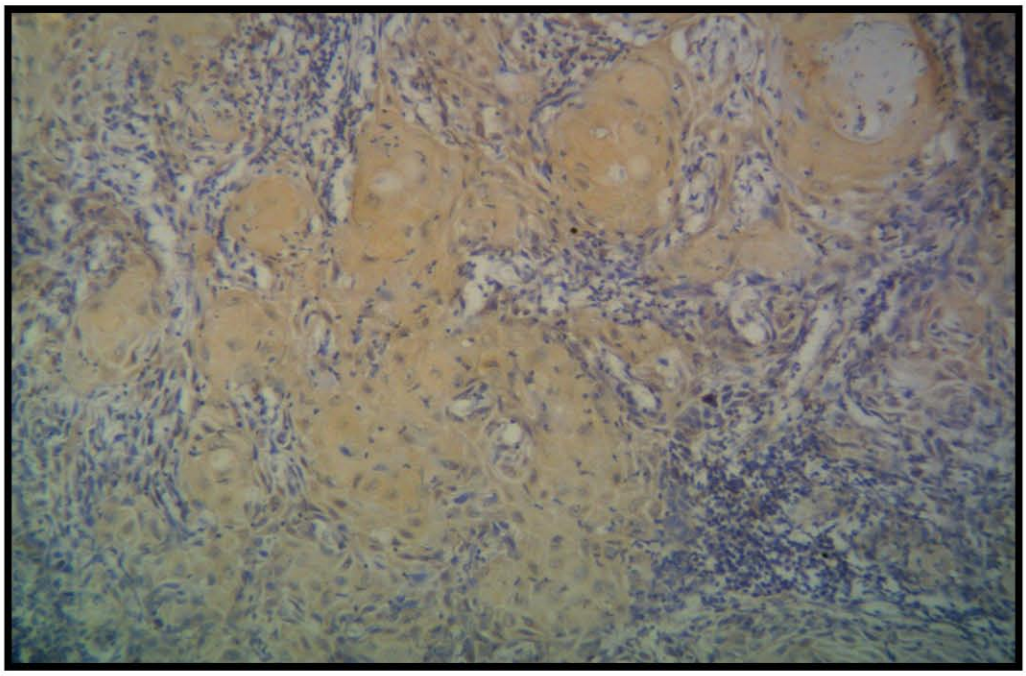
CP 25 : Photomicrograph showing survivin expression of Grade 2+ on Well differentiated squamous cell carcinoma (10x)



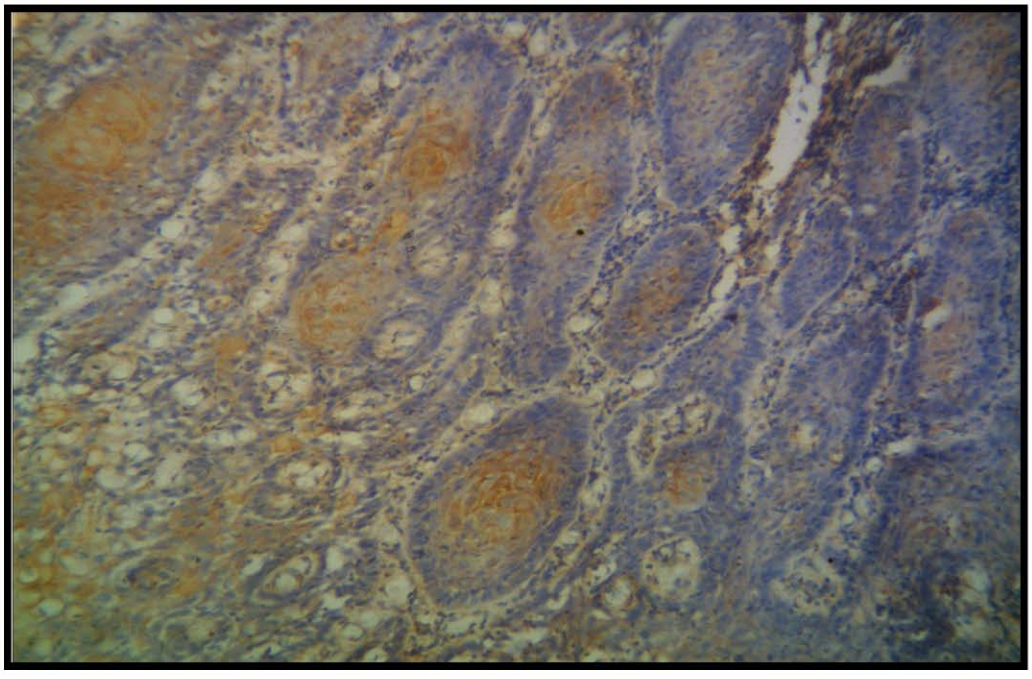
CP 26 : Photomicrograph showing Moderately differentiated squamous cell carcinoma [H&E stained] (10x)



CP 27 : Photomicrograph showing survivin expression of Grade 1+ on Moderately differentiated squamous cell carcinoma (10x)



CP 28 : Photomicrograph showing survivin expression of Grade 2+ on Moderately differentiated squamous cell carcinoma (10x)



DISCUSSION

Oral squamous cell carcinoma is one of the commonest malignant tumors in humans, the development of which includes a number of malfunctions in gene regulation such as activation of oncogenes and inhibition of cancer suppressor genes. Studies by Thompson (1995) suggested that deregulation of apoptosis plays a critical role in the onset and progression of cancer.[103] Singh et al (1988) in his study showed that deregulated Bcl-2 in severely dysplastic oral epithelial lesions was linked to progression of OSCC.[104]

A similar paradigm has also been suggested for other solid malignancies thus prompting the search for additional molecular markers potentially influencing the cell death/cell viability balance in cancer. In this context, recent studies identified survivin, an inhibitor of apoptosis family protein to be over expressed in most human cancers but down regulated in normal tissues. Differently from Bcl-2 family molecules, IAP proteins are thought to block a highly evolutionarily conserved step in cell death by binding and inhibiting terminal effector caspases-3 and 7, thus providing a separate and nonredundant pathway of cell viability in cancer.[1]

Studies in India by Gupta et al in1980 have reported that oral leukoplakia has a high malignant transformation rate of 2.2%. The presence of severe epithelial dysplasia characterised by enlarged nuclei and eosinophilic nucleoli, hyperchromatism, dyskeratosis and aberrant mitoses is suggestive of malignant transformation.[88]

Lo Muzio and his coworkers have shown that survivin is upregulated early during malignant transformation of the oral cavity and that its upregulation is

overwhelmingly associated with precancerous lesions that evolved into full-blown invasive carcinomas.[1] According to the study by Tanaka et al (2003) one third of the oral premalignant lesions examined had survivin protein expression.[79] Considerable evidence suggest that elevated expression of survivin may promote tumorigenesis and in fact survivin is highly expressed in human cancers. (Lu et al 2004[51] & Gianani et al 2001[42])

In present study the immunohistochemical expression of survivin was investigated in 30 cases of oral epithelial dysplasia and 30 cases of oral squamous cell carcinoma and compared it with its expression in normal mucosa. Results were obtained and statistical analysis was done using SPSS software.

In the study by Tanaka et al 37% of oral leukoplakia showed survivin positivity and they suggested that survivin protein accumulation might be an early event during oral carcinogenesis.[79] Lo Muzio et al in their study on oral precancerosis showed a survivin positivity of 33% in epithelial dysplasia that did not progress into malignancy and 94% positivity in dysplasias that evolved into oral squamous cell carcinoma.[1]

In the present study out of the 30 cases of oral epithelial dysplasia there were 07 cases of mild dysplasia, 10 cases of moderate dysplasia and 13 cases of severe dysplasia. Overall percentage of survivin positivity in oral epithelial dysplasia was 93% with 24 cases showing grade-1 expression, 04 cases showing grade-2 expression and 02 cases showing negative expression or grade-0 expression. The percentage of survivin positivity was greater than that reported by Lo Muzio et al (33%)[92] and Tanaka et al (37%)[79] & Jimbu Y et al (44%)[95] but, are inaccordinance with the results

of the studies done by Lin CY et al (97%)[80] & Oluwadayo et al (100%)[81] expression. In our study 02 case of epithelial dysplasia showed negative survivin expression. Lo Muzio et al in his study has reported that 50% of severe dysplasia cases showed a negative survivin expression and those cases did not progress into oral squamous cell carcinoma.[92] However the follow up of patients with oral leukoplakia was beyond the scope of this study. (Tables:1&2, Graphs:1&2, Color plates:14-22)

Kim et al in their study obtained a 100% survivin positivity in oral sqaumous cell carcinoma cell lines in hamster oral carcinogenesis model and showed that OSCC patients with high survivin expression in the tumor mass had a shorter overall survival.[97] Lu Mozio et al (2001) observed 56% survivin expression in oral squamous cell carcinoma.[92] In another study (2003) by the same investigators in oral squamous cell carcinoma and precancerous lesions ,a score of 82.7% positivity was seen in oral squamous cell carcinoma.[1] Studies by Tanaka et al and Yoshinori Jinbu et al both revealed a 58% survivin positive immunostaining in oral squamous cell carcinoma cases.[79, 95]

In our study out of the 30 cases of oral squamous cell carcinoma there were 15 cases of well differentiated and 15 cases of moderately differentiated OSCC respectively. Among that 18 cases showed grade-1 & 12 cases grade-2 expression respectively. The overall survivin positivity in oral squamous cell carcinoma was 100%. This was consistent with the observations of Lo Muzio et al (80%)[1], Lin CY et al (98%)[80] & Oluwadayo et al (96%)[81]. The percentage of survivin positivity in the present study was higher when compared to results of the studies done by Lo Muzio et al (56%)[92], Tanaka et al (58%)[79], Jimbu Y et al (55%)[95] & Khan Z et al (72%).[96] In

our study we observed that the grades of survivin expression increased from well differentiated to moderately differentiated OSCC even though the results were not statistically significant. (Tables: 3&4, Graphs: 3&4, Color plates: 23-28)

Earlier studies by Lu Muzio et al have reported mild expression of survivin in the epithelial cells of normal mucosa.[1] In the study by Tanaka et al all the normal oral mucosa examined showed absence or significant down-regulation of survivin expression and were considered negative.[79] Fukuda S et al in their review on survivin stated that although survivin is expressed and regulated in normal tissues characterised by self-renewal and proliferation, its expression is significantly lower than in transformed cells.[17]

In our study survivin positivity in normal mucosa was noted in 04 cases with grade-1 & grade-2 in one case. Rest 25 cases showed negatiive survivin expression with grade-0 score. The total positivity rate was only 16.7% when compared to negative expression which was 83.3%. The results of this study were in accordance with the studies reported by Lo Muzio et al and Tanaka et al.[1, 79] Normal oral mucosa showed a low survivin expression in comparison with oral epithelial dysplasia and oral squamous cell carcinoma. (Tables: 5&6, Graph: 5, Color plates: 11-13)

There was high statistical significance when the expression of survivin in oral oral epithelial dysplasia and oral squamous cell carcinoma were compared with normal mucosa. When the expression of survivin in OSCC was compared with that in normal mucosa a statistical significance was obtained at 0.001 levels. A comparison of survivin expression among all the three groups (oral epithelial dysplasia, oral squamous cell carcinoma and normal mucosa) obtained a statistical

significance with a P value of 0.001 (significant at P<0.05 level with Kruskal-Wallis test). (Tables: 7,8&9, Graphs: 6,7&8)

In this study the survivin expression and OSCC were significantly higher than that in normal oral tissues. As the data presented here suggest, survivin expression might provide a strong advantage for tumor progression by protecting tumor cells from broad apoptosis-inducing stimuli and by maintaining proper mitotic progression of the proliferating and metastatic population.

SUMMARY & CONCLUSION

The immunohistochemical expression of survivin in 30 cases each of oral squamous cell carcinoma, oral epithelial dysplasia and normal mucosa was investigated in the present study. Results were evaluated and a significant statistical difference was obtained in relation to survivin expression when all the three groups of oral squamous cell carcinoma, oral epithelial dysplasia and normal mucosa were compared.

The data suggests that survivin is upregulated in oral squamous cell carcinoma when compared to that in oral epithelial dysplasia and normal mucosa. The expression of survivin was low in epithelial dysplasia when compared to oral squamous cell carcinoma. But the survivin expression in normal mucosa was least in the present study.

Based on the results it can be concluded that survivin which is an inhibitor of apoptosis protein can be identified as a useful tool for the identification of precancerous lesions at higher risk for progression into invasive carcinoma. Further studies on a larger group or series of patients with follow up may provide more accurate information about the involvement of survivin in the development and progression of oral carcinogenesis.

This study was done in the Department of Oral Pathology and Microbiology, Sree Mookambika Institute of Dental sciences, Kulasekharam to evaluate the immunohistochemical expression of survivin in oral squamous cell carcinoma, oral epithelial dysplasia and normal mucosa.

Immunohistochemical staining was performed in four micrometer thick paraffin sections of 30 cases each of oral epithelial dysplasia, oral squamous cell carcinoma and 30 cases of normal mucosa with the use of survivin monoclonal antibody (PathnSitu). The degree of staining for survivin was evaluated by two oral pathologists to whom the clinical data was unknown.

The results were evaluated and statistical analysis was done using SPSS software. A significant statistical difference was obtained at 0.001 level ($P<0.05$) when all the three groups of oral squamous cell carcinoma, oral epithelial dysplasia and normal buccal mucosa were compared. An increase in survivin expression was seen oral epithelial dysplasia (93%) and oral squamous cell carcinoma(100%) to that of normal mucosa 16.7%. This study supports the view that survivin, an inhibitor of apoptosis protein plays a potential role as an early predictor of malignant transformation in precancerous and cancerous lesions of the oral cavity.

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ANNEXURES

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES

KULASEKHARAM, KANYAKUMARI DIST., TAMIL NADU, INDIA.

INSTITUTIONAL RESEARCH COMMITTEE

Certificate

This is to certify that the research project protocol, ***Ref no: 02/03/2014*** entitled, ***"Immunohistochemical Expression of Survivin in Normal mucosa, Oral Epithelial Dysplasia and Oral Squamous Cell carcinoma: A Comparative Study"*** submitted by ***Dr. George Jacob. II Year MDS, Dept. of Oral Pathology & Microbiology has*** been approved by the Institutional Research Committee at its meeting held on ***18th March 2014.***

Convener
Dr. T. Sreelal

Secretary
Dr. Anuroopa A.

Sree Mookambika Institute of Medical Sciences
Kulasekharam (K.K District, TN) 629161

Phone No: 04651-280866, Fax No. 04651-280740

Institutional Human Ethics Committee

Registered under CDSCO with Reg No. ECR/446/Inst/TN/2013

Ref. No. SMIMS/IHEC/2014/A/27 **Date: 26th May 2014**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2014/A/27,** entitled "Immunohistochemical Expression of Survivin in Normal Mucosa, Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma" submitted by Dr. George Jacob, Postgraduate of Department of Oral Pathology and Microbiology, SMIDS has been approved by the Institutional Human Ethics Committee at its meeting held on 6th of May 2014.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES * KULASEKHARAM * IHEC Date 26/05/2014

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam [K.K District]
Tamil Nadu -629161

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES
Kulasekharam, Kanyakumari District – 629161.

Department of Oral Pathology & Microbiology

BRIEF CASE RECORD PROFORMA

OP. No. : Biopsy No. :

Name:

Age: Sex:

Clinical Details:

Site:

Size:

Clinical Appearance:

Duration:

Diagnosis:

Provisional Diagnosis:

Histopathology:

Final Diagnosis:

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES
Kulasekharam, Kanyakumari District – 629161.

Department of Oral Pathology & Microbiology

PATIENT INFORMATION SHEET

Subject: CANCER RESEARCH USING IMMUNOHISTOCHEMISTRY

Introduction:

A research is being conducted in the Department of Oral and Maxillofacial Pathology about oral cancer. Please read this consent form thoroughly and ask the researcher any queries you may have about the study before signing the consent form. For this purpose the name and phone number of researchers is given below.

If oral cancer is detected earlier, it can be treated and cured to a certain extent. This study is aimed at planning a new technique for the early detection of cancer. Each cell in the human body contains various proteins. Of these, the level of expression of some proteins varies in cancer cells. In this study, we are comparing the variations in the levels of expression of one such protein called survivin in normal, precancerous and cancer cells. This will enable the detection of cancer at a very early stage.

A part of the tissue which was taken earlier from your mouth for diagnosis is kept in the department. If you agree to participate in the study, this bit of tissue will be used for the research. Your participation is very important to the success of this scientific research. If you are interested, kindly sign the consent form and the results of the study will be informed to you later.

Confidentiality:

The information concerning your participation in the study will be kept confidential and used only for scientific purposes. No one except members of the research team will have access to the test results. Your name will not be used in any report or released in any way.

If you have any doubts/queries, please contact:

Dr. George Jacob: 08056363290; Dr. Girish. KL: 09611835503.

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES
Kulasekharam, Kanyakumari District – 629161.

DEPARTMENT OF ORAL AND MAXILLOFACIAL PATHOLOGY

CONSENT FORM

I ---------- am aware of the "Immunohistochemistry" research project being conducted at Sree Mookambika Institute Of Dental Sciences, Kulasekharam, after reading the patient information sheet. I have been assured by the doctor that all details regarding this research will be kept confidential and if interested the result of the study will be informed. No pressure has been put on me to participate in this research. I am voluntarily willing to participate in this research project.

Patient's name: Name of witness:

Signature Signature

Address Address

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES
Kulasekharam, Kanyakumari District – 629161.

Department of Oral Pathology & Microbiology

DATA ENTRY SHEET

IMMUNOHISTOCHEMICAL EXPRESSION OF SURVIVIN IN NORMAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

Block no : NN/YY

Enroll no : SUR 14 XXX

Provisional diagnosis :

Histopathological diagnosis :

Histopathological Grading :

Mild dysplasia	
Moderate dysplasia	
Severe dysplasia	
Well differentiated SCC	
Moderately differentiated SCC	
Poorly differentiated SCC	

IHC staining with Survivin :

Grade :

Negative expression	
Weak expression	
Strong expression	

தகவல் அட்டவணை

பொருள் : இமுனோகிஸ்றோகெமிஸ்ரி பயன்படுத்தியுள்ள புற்றுநோய் ஆய்வு

முன்னுரை :

வாயில் புற்றுநோய் எவ்வாறு வருகிறது என்பதை குறித்து ஓரல் அண்ட் மேக்சிலோபேஷியல் பேதாளஜி டிப்பாட்மேன்றில் ஆராய்ச்சி நடக்கிறது. கீழே குறிப்பிட்டுள்ளவற்றை நன்கு படித்து, சந்தேகம் இருந்தால் மருத்துவரிடம் ஆலோசனை கேட்டபின்பு உறுதிபத்திரத்தில் கையொப்பம் இடவும். (இதற்கு வேண்டி மருத்துவரின் தொலைபேசி எண், மற்றும் முகவரி இதனுடன் இணைக்கப்பட்டுள்ளது.

ஆரம்ப நிலையிலே புற்றுநோய் கண்டுபிடித்தால் ஓர் எல்லைவரை மிக எளிதில் குணமாக்கலாம். ஆரம்பத்திலே புற்றுநோயை கண்டுபிடிப்பதற்கான ஓர் புதிய வழியே இவ் ஆராய்ச்சி ஆகும். மனித உடம்பில் உள்ள செல்களில் பலவிதமான புரோட்டீன்கள் உண்டு. புற்றுநோய் உண்டான செல்களில் இப்புரோட்டீன்களின் அளவு வேறுபடும். சர்வைவின்ற என்னும் புரோட்டீன் அளவு ஆரோக்கியமான திசுகளிலும், புற்றுநோய்யால் பாதிக்கப்பட்ட திசுகளிலும் எவ்வளவு சதவீதம் வேறுபட்டுள்ளது என்பதை குறித்துதான் இவ் ஆய்வு.

உங்களுடைய வாயில் புற்றுநோய் பாதிக்கப்பட்ட இடத்தி ருந்து எடுக்கப்பட்ட திசுபாகம் எங்களுடைய டிப்பாட்மேன்றில் வைக்கப்பட்டுள்ளது. நீங்கள் சம்மதித்தால் மட்டும், எங்கள் டிப்பாட்மேன்றில் வைத்துள்ள திசுவை நாங்கள் ஆராய்ச்சிக்காக பயன்படுத்துவோம். இந்த ஆராய்ச்சிக்கு உங்களுடைய பங்களிப்பு மிகவும் முக்கியமானதாகும். இவ் ஆராய்ச்சியின் முடிவை அறிவிப்பது, உங்களுடைய மனவ மையை சார்ந்தே ஆகும்.

இரகசியமான பாதுகாக்கப்படும் என உறுதி :

நீங்கள் இந்த ஆராய்ச்சியில் பங்கெடுத்த விவரத்தை நாங்கள் இரகசியமாக வைத்திருப்போம். இந்த விவரங்கள் அறிவியல் ரிதியான முறையில் மட்டுமே

பயன்படுத்தப்படும். இந்த ஆராய்ச்சியின் முடிவு, ஆராய்ச்சிக்கு குழுவினரை தவிர வேறுயாருக்கும் தெரிவிக்கப்படாது. ரிப்போர்ட்டில் உங்களுடைய பெயர் எழுதபடாது.

கேள்விகளுக்கும், பிரச்சனைகளுக்கும் அணுகவும் :

இந்த ஆராய்ச்சிக்கு உங்களுடைய பங்களிப்பு மிகவும் முக்கியமானதாகும். இவ் ஆராய்ச்சியைக் குறித்து ஏதேனும் சந்தேகம் இருந்தால் கீழே கொடுத்துள்ள மருத்துவரை அணுகவும்.

மருத்துவரின் பெயர் :

முகவரி :

தொலைபேசி எண் :

டிப்பார்ட்மெண்ட் ஆப் ஓரல் அண்ட் மேக்சிலோபேஷியல் பேதாளஜி

ஒப்பந்தம்

நான் ..... ஸ்ரீ மூகாம்பிகா இன்ஸ்டிடூட் ஆப் டென்டல் சைன்ஸில் நடக்கின்ற இமுனோகிஸ்றோகெமிஸ்ரி ஆராய்ச்சியை பற்றியுள்ள தகவல்களை நன்கு அறிந்துள்ளேன். இவ் ஆராய்ச்சியின் முடிவை இரகசியமாக வைக்கப்படும் என்றும், நான் விருப்பப்படும் போது மட்டும் முடிவை என்னிடம் தெரிவிக்கப்படும் என்று மருத்துவர் உறுதியளித்து இருக்கிறார். இந்த ஆய்வில் நான் பங்குபெற யாரும் என்னை வற்புறுத்தவில்லை. இந்த ஆராய்ச்சியில் நான் பங்குபெற மனப்பூர்வமாக ஒப்புக்கொள்கிறேன்.

நோயாளியின் பெயர் : சாட்சியின் பெயர் :

கையொப்பம் : கையொப்பம் :

முகவரி : முகவரி :

மருத்துவரின் கையொப்பம் :

டிப்பாட்மென்ட் ஆப் ஓரல் அண்ட் மேக்சிலோபேஷியல் பேதாளஜி

ஸ்ரீமூகாம்பிகா இன்ஸ்டிடூட் ஆப் டென்டல் சைன்ஸ்

குலசேகரம், கன்னியாகுமரி மாவட்டம்.

വിവരണ പത്രിക

വിഷയം: ഇമ്മ്യൂണോഹിസ്റ്റോകെമിസ്ട്രി ഉപയോഗിച്ചുള്ള അർബുദ ഗവേഷണം

ആമുഖം

വായിലെ അർബുദത്തെക്കുറിച്ച് ഓറൽ ആന്റ് മാക്സില്ലോഫേഷ്യൽ പതോളജി വിഭാഗത്തിൽ ഗവേഷണപഠനം നടക്കുന്നു ്. താഴെപ്പറയുന്ന കാര്യങ്ങൾ വിശദമായി വായിച്ചശേഷം, സമ്മതപത്രത്തിൽ ഒപ്പിടുന്നതിനുമുമ്പ് പഠനത്തെ സംബന്ധിച്ച് താങ്കൾക്കുള്ള ഏതു സംശയവും ഡോക്ടറോട് ചോദിച്ച് മനസ്സിലാക്കാവുന്നതാണ്. ഇതിനു വേ ി ഡോക്ടറിന്റെ ഫോൺ നമ്പറും അഡ്രസ്സും ഇതിനോടൊപ്പം ചേർത്തിട്ടു ്.

പ്രാരംഭദിശയിൽത്തന്നെ രോഗം നിർണയിക്കുവാൻ സാധിച്ചാൽ അർബുദത്തെ ഒരു പരിധിവരെ ചികിത്സിച്ചു ഭേദമാക്കാവുന്നതാണ്. പ്രാരംഭദിശയിൽത്തന്നെ രോഗം നിർണയിക്കുവാനുള്ള ഒരു പുതിയ മാർഗ്ഗം ആവിഷ്കരിക്കുന്നതിനുവേ ിയാണ് ഈ പഠനം നടത്തുന്നത്. മനുഷ്യശരീരത്തിലെ കോശങ്ങളിൽ വിവിധയിനം മാംസ്യങ്ങൾ (പ്രോട്ടീൻ) ഉ ്. അർബുദം ബാധിച്ച കോശങ്ങളിൽ ഇവിയിൽ ചില മാംസ്യങ്ങളുടെ തോതിൽ വ്യതിയാനമു ാകും. ഇവയിലുൾപ്പെടുന്ന സർവൈവിൻ എന്ന മാംസ്യത്തിന്റെ അളവ് ആരോഗ്യമുള്ള ദശയിലും അർബുദം ബാധിച്ച ദശയിലും എത്രമാത്രം വ്യതിയാനപ്പെട്ടിരിക്കുന്നു എന്നതാണ് ഈ ഗവേഷണത്തിൽ പഠനവിധേയ മാക്കുന്നത്.

താങ്കളുടെ വായിൽ നിന്നും നേരത്തെ രോഗനിർണ്ണയത്തിനായി മുറിച്ചെടുത്തിട്ടുള്ള ദശയുടെ ഒരു ഭാഗം ഡിപ്പാർട്ട്മെന്റിൽ സൂക്ഷിച്ചിട്ടു ്. തങ്ങാകൾ ഈ പഠനത്തിൽ പങ്കെടുക്കാൻ സമ്മതിക്കുന്ന പക്ഷം സൂക്ഷിച്ചു വച്ചിരിക്കുന്ന ഈ ദശയാണ് ഗവേഷണത്തിനായി ഉപയോഗിക്കുന്നത്. ഈ പഠനത്തിന്റെ വിജയത്തിന് നിങ്ങളുടെ പങ്കാളിത്തം വളരെ പ്രധാനപ്പെട്ടതാണ്. നിങ്ങൾ താൽപര്യപ്പെടുന്ന പക്ഷം പഠനത്തിന്റെ ഫലം അറിയിക്കുന്നതാണ്.

രഹസ്യ സ്വഭാവം സംബന്ധിച്ച ഉറപ്പ്

താങ്കൾ ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതു സംബന്ധിച്ച വിവരങ്ങൾ രഹസ്യമായി സൂക്ഷിക്കുന്നതാണ്. ഈ വിവരങ്ങൾ ശാസ്ത്രസംബന്ധമായ കാര്യങ്ങൾക്കു മാത്രമേ ഉപയോഗിക്കുകയുള്ള. പഠനസംഘത്തിലെ അഗങ്ങൾക്ക് അല്ലാതെ മറ്റാർക്കും ഗവേഷണഫലങ്ങൾ അറിയാൻ സാധിക്കുകയില്ല. റിപ്പോർട്ടുകളിലൊന്നും നിങ്ങളുടെ പേര് ഉപയോഗിക്കുന്നതല്ല.

ചോദ്യങ്ങൾക്കും പ്രശ്നങ്ങൾക്കും സമീപിക്കുക

_____ഈ സുപ്രധാന ഗവേഷണ പദ്ധതിയിൽ നിങ്ങളുടെ സഹകരണം ഞങ്ങൾ വിലമതിക്കുന്നു. ഏതെങ്കിലും വിധതിതലുള്ള സംശയങ്ങൾ ഈ പഠനത്തെക്കുറിച്ച് ഉ ാകുന്ന പക്ഷം, താഴെ പറയുന്നവരുമായി ബന്ധപ്പെടുക.

ഡോക്ടറുടെ പേര് :

അഡ്രസ്സ്:
ഫോൺ :

ഡിപ്പാർട്ടുമെന്റ് ഓഫ് ഓറൽ ആന്റ് മാക്സില്ലോഫേഷ്യൽ പതോളജി

സമ്മതപത്രം

ഞാൻ,_____..ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസിൽ നടക്കുന്ന ഇമ്മ്യുണോഹിസ്റ്റോകെമിസ്ട്രി പഠനത്തെപ്പറ്റി വിവരണപത്രിക വായിച്ചതിൽ നിന്നും ബോധവാനാണ്. ഈ പഠനത്തിൽ ഞാൻ പങ്കെടുക്കുന്നതു സംബന്ധിച്ച വിവരങ്ങൾ രഹസ്യമായി സൂക്ഷിക്കുന്നതാണെന്നും താൽപര്യപ്പെടുന്ന പക്ഷം പഠനത്തിന്റെ ഫലം അറിയിക്കുന്നതാണെന്നും ഡോക്ടർ ഉറപ്പു തന്നിട്ടു ്. ഇതിൽ പങ്കെടുക്കുന്നതിനായി എന്റെമേൽ യാതൊരു വിധത്തിലുള്ള പ്രലോഭനങ്ങളും സന്മർദ്ധങ്ങളും ഉ ായിട്ടില്ല. ഈ സാഹചര്യത്തിൽ പഠനപദ്ധതിയുടെ ഭാഗമാകുന്നതിന് എനിക്ക് പൂർണ്ണ സമ്മതമാണ്.

രോഗിയുടെ പേര് :

ഒപ്പ്:

വിലാസം:

സാക്ഷിയുടെ പേര്:

ഒപ്പ് :

വിലാസം:

ഡോക്ടറുടെ പേര്:

ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ ആന്റ് മാക്സില്ലോഫേഷ്യൽ പതോളജി ,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
കുലശേഖരം, കന്യാകുമാരി ഡിസ്ട്രിക്റ്റ്.